ORAL PRESENTATIONS
COMMUNICATIONS ORALES

Postmortem toxicology
Toxicologie postmortem

Understanding forensic toxicology in relation to external-cause deaths

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AIMS: Injuries are not only recognised as an important public health problem, but are also one of the major causes of death. Injuries accounted for 9% of the world's deaths in 2000 and 12% of the world's burden of disease (Peden, M, McGee, K, et al (2002). The Injury Chart Book: a graphical overview of the global burden of injuries). It is known that drug-drug and/or drug-alcohol interactions cause an increased risk of mortality. The use of such mind-altering drugs in places of employment or by drivers of motor vehicles, for example, places the individual and other members of the community at risk. However, the full extent of the involvement of drugs across the whole range of injury deaths is mostly unknown. Illegal drugs are more likely to be the cause of unintentional death than intentional. In contrast, in Australia, pharmaceuticals are more likely in self-harm, where analgesics and psychoactive drugs appear to be most commonly responsible for poisoning and/or suicide (Drummer OH & Odell M (2001). The Forensic Pharmacology of drugs of abuse. Chapter1.1: pp2-3).
The study aimed to examine the presence and contribution of alcohol and drugs in all external cause deaths for the period 2000 to 2005 in Victoria, Australia (population 20.7 million). A secondary aim was to use the research results to assist with improving the National Coroners Information System (NCIS) as a tool for alcohol and drug injury surveillance.

METHODS: The extent to which drugs and alcohol contribute to unnatural deaths can now be described at a population level for the first time in Australia using the NCIS (a national database of coronial information). Associated toxicology reports were examined to determine the proportion of cases that contained alcohol and drugs and the type and range (therapeutic, supra-therapeutic and toxic) of drugs.

RESULTS: There were 7,673 external cause deaths in Victoria between July 2000 and June 2005. Of these, there were 2,086 (27%) external cause deaths contained a toxicology report where alcohol was identified as positive (greater than 0). A toxicology report was not attached in 1,420 (18.5%) cases and the remaining 4,152 external cause cases contained a negative result for alcohol. Of cases with a toxicology report 455 (5.4%) had a blood alcohol concentration (BAC) of less than 0.06 g/100 mL. There were 564 (7.4%) detected with alcohol (0.06 g/100mL to <0.16 g/100mL) and 659 (8.6%) had a toxic level of alcohol (>0.16 g/100 mL). Of all causes with alcohol detected, 2726 (37%) were due to intentional self-harm, followed by 2005 (27%) being transport related, 932 (12%) due to poisoning and 553 (7%) being fall related. The trends and themes of other drugs will also be reported especially in relation to traffic injuries.

CONCLUSIONS: The study results provide, for the first time in Australia, a systematic examination of the epidemiology of licit and illicit drugs in injury deaths due to all mechanisms.

Buprenorphine-related deaths: low levels may be significant

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AIMS: Buprenorphine is a partial mu-opioid receptor agonist, and is used for the treatment of moderate to severe chronic pain, but has also been introduced as an alternative to methadone in opiate substitution therapy. Some years after its introduction on the Swedish market, illegal use of buprenorphine started, and several deaths involving buprenorphine have been documented in Sweden. The aim of this study was to explore the femoral blood and urine levels in buprenorphine-positive cases, and to compare these concentrations with those observed in driving under the influence cases (DUI).

METHODS: A slightly modified previously published LC-MS/MS method (Kronstrand et al, JAT 2003;27:464-70) was used to determine buprenorphine on both postmortem cases and DUI cases where subjects either were prescribed buprenorphine, or when abuse was suspected.

RESULTS: The mean ± SD buprenorphine concentrations in postmortem femoral blood (all cases, n = 32) and in blood from DUI cases (n = 94) were 3.1 ± 4.7 and 1.6 ± 1.8 ng/g, respectively. The overlap was substantial both for buprenorphine and norbuprenorphine levels. Additionally, in four postmortem cases, where intoxication was ruled out (causes of death: hanging = 2, pulmonary embolism = 1 and severe liver cirrhosis =

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1), the mean (median) concentration was 7.8 (4.3) ng/g. Hence it seems impossible to define a fatal level. It may be argued that buprenorphine was actually not involved at all in any of these deaths, explaining these toxicological results. However, in at least eleven of the intoxication cases, buprenorphine was apparently the most suspected drug based on circumstances, and the yet the mean ± SD were only 2.0 ± 2.0. Further, these subjects typically presented with massive pulmonary edema with lung weights averaging 1394 ± 312 g as compared to 1142 ± 297 g in postmortem controls (hangings, n = 1,979), and often with froth in the airways, suggesting that an overstimulation of the mu-opioid receptors in the brain stem resulting in respiratory depression as the basic mechanism for their demise. No other opioid drugs were present in their blood. The ratio norbuprenorphine to buprenorphine in postmortem cases (mean 1.15) was similar to that in DUI cases (mean 1.55), but in 20 of the postmortem cases the buprenorphine concentration was higher than that of norbuprenorphine suggesting recent intake. In addition, urine analysis indicated a period of abstinence before the last dose.

CONCLUSIONS: In eleven postmortem cases effects of buprenorphine was considered the main cause of death even though the blood concentrations were comparable to those of DUI cases and postmortem controls. This corroborates the notion that interpretation of postmortem levels of opioid drugs warrants caution and comprehensive review of each case.

A fatality occurring during tumescent liposuction

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CASE REPORT: Over the past decade, liposuction has become the most frequently performed procedure in aesthetic plastic surgery in many countries although it should not be considered without complications. Tumescent local anesthesia is subcutaneous infiltration of very dilute lidocaine and epinephrine to produce subcutaneous swelling. Mepivacaine has never been mentioned to be used in this kind of surgery. We report a case of overdose with mepivacaine and lidocaine in a 38-year-old patient who died during a procedure of tumescent liposuction of abdomen and both thighs in an outhospital clinic. According to one witness, the victim suffered an episode of tonic-clonic convulsion. When the emergency medical services arrived the patient was under cardiac arrest and the cardiopulmonary resuscitation measures were of no use.

METHODS: All drugs involved in the case were detected using gas chromatography with nitrogen-phosphorus detector and confirmed using gas chromatography-mass spectrometry full scan mode after solid-phase extraction using Chem-Elut columns. An additional high-performance liquid chromatography coupled to diode-array detection screening also obtained the same results. Quantitative analysis of the two local anaesthetics, lidocaine and mepivacaine, was undertaken by GC-NPD by comparison of each peak-area ratio with that of the IS against blood calibration curves (1, 5, and 10 mg/L). Limits of detection were 0.05 mg/L, the upper limit of linearity was 10 mg/L, accuracy was > 95%, and precision (n=3) demonstrated CV's < 7% at 5 mg/L for both compounds.

RESULTS: Autopsy results showed general congestion with no specific signs of anaphylactic shock. Toxicological analysis revealed the presence of lidocaine and mepivacaine in heart blood, at concentrations of 4.9 and 16.2 mg/L, respectively. There are no defined anatomic or microscopic markers for toxicity caused by local anesthesia and toxicology remains the diagnostic mainstay.

CONCLUSIONS: Based on the autopsy findings, case history, and toxicology results, the forensic pathologists ruled that the cause of death was due to an overdose of local anesthetic agents. The low complication rates achieved in lipoplasty are due to adequately trained surgeons and anesthesia providers, and the diligent intraoperative and postoperative monitoring, which were mostly neglected by the operating surgeon involved in our case. Therefore the manner of death was considered accidental although due to gross medical negligence.

Dyadic death – an unusual family tragedy

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CASE REPORT: This report involves the death of two women (mother and daughter) in unusual circumstances. A 15 year old girl (Ms A) had stayed overnight at the residence of a 26 year old man thought to be her boyfriend. The boyfriend claimed he located the young girl deceased in his room when he awoke at about 10:00 a.m. the next morning. The boyfriend was also an acquaintance of the mother (41Y) of the young girl. The young girl's brother was also at the boyfriend's residence but had returned to his mother's residence late in the evening as he had to work the next day. The brother mentioned that when he left for work early that morning he heard his mother snoring lightly, but did not disturb her. He stated when he returned home from work his mother was still in bed where he had seen her in the

morning. Upon checking her he found her deceased. The mother suffered from depression and was prescribed a number of drugs including fluoxetine, mirtazapine and citalopram.

METHODS: Both cases were subject to a full autopsy and toxicological examination. This involved an immunoassay urine/blood screen for amphetamines, benzodiazepines, cannabinoids, cocaine metabolites and opioids. Blood extracts were also analysed on a capillary gas chromatographic screen using a nitrogen-phosphorus detector for basic and neutral drugs as well as an additional screen conducted by gradient elution high performance liquid chromatography. Drugs of significance were quantified using either HPLC/DAD or GC/MS. Further tests for alcohol and other volatiles were separately conducted.

RESULTS: Pathological findings following the autopsy of the young girl showed no significant natural disease, there was some pulmonary oedema, but no other findings of note. There was no significant natural disease or injury detected in the mother following autopsy. The concentrations of drugs detected in the young girl are consistent with death from mixed drug toxicity (see table below) including recent heroin use. Citalopram, fluoxetine and diazepam detected in the young girl were prescribed for the mother. The mother also had very similar drugs in her blood. The concentration of morphine (free) 10 mg/L detected in the mother's blood is extremely high – despite concerns regarding postmortem redistribution, postmortem metabolism, degradation of glucuronides, tolerance and pharmacogenetic differences between individuals, it is difficult to ignore the concentration of morphine and its contribution to death.

CONCLUSIONS: In our opinion the use of this drug is consistent with excessive, intentional consumption and combination with the other drugs detected and is likely to have caused this woman's death. It is remarkable that the drugs found in the mother are in keeping with those found in her daughter. Over the 4 months to the deaths the boyfriend had been prescribed morphine from a variety of doctors at different practices. During the inquest the prescribing doctor spoke about the perennial problem of doctors being unable to obtain access to a real time data base of an individual's prescription history due to privacy issues. The coroner deemed that both mother and daughter were not the victims of "foul play", and the overdose (in each case) was accidental. The coroner also gave consideration to tightening the current "doctor shopping" guidelines to provide an earlier alert system. A mechanism for introducing a cross referenced system for pharmacies to notify the doctor shopping service of over prescribed patients.

Ms A* (daughter)		Mother*	
Drug	Concentration	Drug	Concentration
Methamphetamine	0.05 mg/L		
Methamphet. (urine)	Detected		
Morphine, free	0.1 mg/L	Morphine, free	10 mg/L
Morphine, total (urine)	Detected >15 mg/L		
6-AM (urine)	0.03 mg/L		
Citalopram	1.0 mg/L	Citalopram	4.4 mg/L
Fluoxetine	0.8 mg/L	Fluoxetine	0.2 mg/L
Diazepam	0.1 mg/L	Diazepam	0.1 mg/L
Nordiazepam	0.1 mg/L	Nordiazepam	0.1 mg/L
Δ^9-THC	4 ng/mL	Δ^9-THC	4 ng/mL
		Mirtazapine	0.6 mg/L
		Valproic Acid	16 mg/L

**Femoral blood results unless otherwise specified; alcohol was not detected (<0.01 g/100 mL) in either deceased.*

The role of methamphetamine in cause and manner of death – an update

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AIMS: The aim of this study is to examine the role of methamphetamine in deaths for four counties in Washington State between 2000 and 2005.

METHODS: Counties were selected for the accessibility of their death investigation records in electronic format and the fact that they are among the most populous, comprising 60% of the State's population.

RESULTS: In 489 cases where the presence of methamphetamine was confirmed toxicologically, we retrieved and reviewed medical examiner determinations of the cause and manner of death. Of these cases, 337 (69%) of the cases were determined to be non-drug-caused, where the presence of methamphetamine might be either a contributory or incidental finding. For example, in the methamphetamine-present-non-drug-caused cases, the most common manner of death was suicide (25%), followed by homicide (23%), accident-traffic (18%), accident non-traffic (17%), and natural (12%).

152 (31%) of the deaths were determined to be drug caused. Of these drug-caused deaths, 97% were reported as being accidental, and < 1% suicidal. The median methamphetamine concentration in drug-caused cases was 0.35 mg/L (range 0.01 - 34.64 mg/L, mean 1.53 mg/L), and was the same as in the non-drug caused deaths. These similarities emphasize the fact that blood methamphetamine concentrations in and of themselves are insufficient to make a cause of death determination. The highest median value of methamphetamine was among deaths ruled as accident-traffic-non-drug-caused. In this group the median value was 0.65 mg/L (range 0.02 - 3.72 mg/L, mean 0.83 mg/L). This median value is higher than that from a prior study of methamphetamine fatalities (Logan et al., 1998), which found a median of 0.35 mg/L for accident-traffic-non-drug-caused deaths, with a range of 0.05 - 2.60 mg/L. Among the 152 drug-caused deaths, the median concentration of methamphetamine was significantly higher ($p < 0.05$) among methamphetamine-only cases (median 0.52 mg/L, s.d. 3.67, $n = 27$) compared to decedents for whom another CNS active drug was detected in addition to methamphetamine (median 0.3 mg/L, s.d. 4.52). This suggests that mortality risk from lower levels of methamphetamine is increased when it is combined with other drug use.

CONCLUSIONS: Our data supports other literature that suggests that the behavior of individuals using methamphetamine predisposes them to greater risk of injury and assault, and the socioeconomic consequences of drug use, together with the depression associated with drug withdrawal may contribute to suicidal ideation.

Death and brain injury from an apparent intentional methomyl poisoning

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AIMS: Pesticide poisoning is a common cause of morbidity and mortality worldwide but is rarely encountered in the death investigation toxicology laboratory in the United States. Methomyl (Acinate, Agrinate, DuPont 1179, NuBait,) is a highly toxic, class 1 carbamate insecticide which has been in use with restrictions since 1970. Like all carbamates, methomyl inhibits cholinesterase activity and results in symptoms of diarrhea, nausea and vomiting, abdominal pain, excessive sweating and salivation, blurred vision, difficulty breathing, headache and muscular fasciculation, leading to respiratory arrest and death. We present here a case of an apparent double methomyl poisoning resulting in one fatality, and one non-fatal case of anoxic brain injury.

CASE HISTORY: A male subject and his female companion were drinking together when he suddenly vomited, collapsed, and subsequently died. His companion developed blindness and confusion and was discovered approximately 22 hours later in her apartment. She was seemingly unaware that the male victim was deceased on the floor in front of her couch. Autopsy results in the male victim indicated early decomposition and mild to moderate coronary artery disease, but no obvious anatomic cause of death. The female victim was diagnosed with stroke as a result of oxygen deprivation. Investigators initially suspected methanol or ethylene glycol poisoning based upon the female victim's symptoms.

METHODS: Blood from both victims was analyzed for volatiles by headspace gas chromatography and headspace GC-MS. Drug screening was performed for cocaine, opiates, methadone, benzodiazepines, PCP, amphetamines, barbiturates, tricyclic antidepressants, cannabinoids and propoxyphene by EMIT, and for basic and acid-neutral drugs by GC-MS. No drugs other than caffeine were detected in either victim. There was a small amount of ethanol (0.02 gm/100 mL) in the male victim's blood. A sample of gastric contents from the decedent was extracted which indicated the presence of methomyl in the basic fraction. It has a molecular weight of 105 amu, and the molecular ion is the most abundant

ion, with two major fragments of 88 m/z and 58 m/z. There was no indication of methomyl in either of the victims' blood or in a swab of the decedent's vomitus. Analysis of other evidence from the scene confirmed the presence of methomyl in two drinking glasses, and in the liquid from the Jaegermeister bottle.

RESULTS AND CONCLUSIONS: Methomyl detection is challenging as it is unstable at standard GC injection port temperatures. In this case, we were able to detect it only due to the high concentrations in the drinks and the gastric content of the decedent. In this case the gastric contents were the only biological sample which tested positive for methomyl, highlighting the benefit of comprehensive postmortem sampling.

Worldwide trends in impaired driving
Conducteurs alcoolisés : les grandes tendances

Alcohol and drug impaired driving in the UK: recent trends and future prospects

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Recent trends in drink driving in the UK have shown an increase in the level of driver fatalities over the 0.08% limit. This is almost certainly related to a decreasing level of traffic enforcement and an associated reduced level of roadside breath testing.

The latter trend has also seen a substantially increased level of those drivers testing positive for alcohol at the roadside over the past 5 years. It needs to be established whether this trend is due to a 'real' increase in the incidence of drink driving or better targeting of offenders.

Legislation was enacted in 2005 to allow for roadside evidential breath testing, but thus far no devices have received government approval for police use. It is expected that such approval will be authorised during 2008. The UK government is committed to providing central funding for such devices, rather than requiring individual police forces to find funds from limited resources.

In a parallel initiative, the development of roadside screening devices which record breath alcohol concentration as well, as personal and demographic data, is also being encouraged for UK use. Such devices would allow a much clearer picture of the extent of drink driving, including those driving with positive alcohol levels below the current UK drink drive level. This data will be important in providing data for a new UK consultation on reducing the limit to 0.05%, due later in 2007.

The UK government is committed to providing central funding for such devices, rather than requiring individual police forces to fund this initiative. Both actions are aimed at tackling an increasing trend in drink driving over recent years.

On the drug driving scene, despite legislation in 2003 allowing for drug screening devices to be used at the roadside, no such devices have yet received government approval. This is particularly disappointing as there is increasing evidence that drug driving is a significant problem in the UK, and many other countries seem to be having increased success in addressing this issue.

These trends and initiatives in drink and drug driving will be discussed together with options for progressing UK policy in near future.

Alcohol, drugs, and traffic safety in Australia: initiatives and indicators

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This paper reviews the status of alcohol, drugs and traffic safety in Australia. Australian jurisdictions have made impressive improvements in road safety since the early 1970s. Road fatalities have more than halved, while indicators of road use (population, vehicle registration, driver licensure, vehicle distance driven) have more than doubled. Enforcement and public education campaigns that specifically target drink driving, speeding, and non-use of seat belts have been successful. There has been an extensive shift in attitudes to drink driving in Australia, and success in this area is serving as a valuable guide to changing other undesirable road behaviours. Strategies to tackle drivers impaired by alcohol or other drugs are based on general deterrence and targeted operations. These actions reflect the national road safety strategy and its analogues across the Australian jurisdictions.

A recent major initiative introduced in a number of Australian jurisdictions is random roadside drug testing, which supports and extends the previous random breath test (RBT) powers for impaired driving. Police now have powers to: (a) stop drivers at random to test for alcohol and arrest drivers who test over the legal prescribed limit; (b) stop drivers at random to test for specified drugs and issue a traffic notice or court attendance notice if certain prescribed drugs are detected; (c) require a driver to undergo a sobriety test in certain circumstances, and arrest drivers they believe are impaired by drugs for the purpose of blood and urine testing. Random roadside drug testing supports an offence of drive with the presence of any of the following drugs: active THC (cannabis); methylamphetamine ('speed/ice'); or methylenedioxymethamphetamine (MDMA or 'ecstasy'), as determined by testing in oral fluid, blood or urine. As well, the presence of morphine (unless proven for medicinal use) and cocaine in the blood or urine of drivers can also constitute an offence. The

usual penalties for drug driving include a substantial fine and loss of driver's license.

Other major initiatives to combat impaired driving in Australia in recent years include responsible service of alcohol programs, and the commencement of alcohol ignition interlock programs and interventions targeting repeat drink driving offenders for assessment of alcohol-dependence. Promotion of the use of personal alcohol breathalyzer devices is also occurring. Support for interventions targeting first-time drink driving and drug driving offenders is lagging, however, despite a stated need for more effective partnerships to be built between the road safety and health sectors to better address issues involving alcohol and other drug use. Some technologies, notably dataloggers and vehicle tracking through GPS, offer promise for a better dealing with impaired drivers, but there has been little policy development to date.

Across the Australian jurisdictions there are now an agreed suite of drink driving and drug driving performance indicators, including: incidence of alcohol and drug use by drivers and riders killed in crashes; crash incidence during 'high alcohol' periods; incidence of drink driving reported from RBT operations; incidence of illegal alcohol and drug levels per 1,000 tests; community perspectives of the level of RBT enforcement and likelihood of being tested; and number of offenders and non-offenders using alcohol interlocks.

Drink-driving trend in the Netherlands; need for a new government policy

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This paper presents the drink-driving trend during autumn/fall weekend nights in the Netherlands as well as the trend of the alcohol-related road toll. Since 1970, annual roadside surveys have been conducted. The objective of these surveys is to obtain an insight into the patterns of drink-driving and into the effect of measures. Road side survey sessions are distributed over Friday and Saturday nights, and random breath testing is performed between 10 pm and 4 am, collecting data from about 30,000 motorists, annually. Breath testing is performed by police officers. Since random breath testing by the police is legally permitted in the Netherlands, non-response is virtually non-existent.

Between 2000 and 2004 a large case-control study was conducted in the Netherlands to assess relative injury risk of driving under the influence of psychoactive substances. The case sample consisted of seriously injured car drivers who were admitted to the Accident Emergency department of the St. Elisabeth Hospital in the city of Tilburg. Controls consisted of motorists who were taken at random from moving traffic in the Tilburg police district which is located in the hospital catchment area. Body fluids (blood and urine) of both cases and controls were tested for alcohol, and a number of licit and illicit drugs. The relative risk of these psychoactive substances was determined by calculating odds ratios. Furthermore, data on publicity campaigns, rehabilitation programmes, medical examinations, and crash data was collected and analyzed.

Between 1970 and 1999 the proportion of drivers with a BAC above 0.5 g/L in weekend nights dropped from 15% to 4.3%. Reductions in this downward trend occurred mainly after police enforcement was intensified. After a period with a more or less stable proportion of illegal BACs, a new decline could be observed since 2003. However, this decline was solely visible at relatively low BACs (≤ 1.3 g/L). Despite efforts of police and government, the proportion of the highest BAC offenders (> 1.3g/L) did not decrease over the past six years.

Results from the case-control study in Tilburg and surroundings indicate that this category is responsible for about three quarters of all alcohol related severe injury crashes.

The declining trend of drink-driving offenders is not reflected in the trend of registered alcohol related fatalities. Estimations of the proportion of alcohol-related road toll which are based on both the results of the annual road side surveys and the results of the case-control study in Tilburg, indicate a more or less stable proportion of alcohol related fatalities over the period from 1999 to 2005.

A governmental policy is needed to lower the alcohol-related road toll significantly. Police enforcement should be targeting high BAC drivers and effective measures should be introduced such as alcolock programmes including alcoholism treatments for alcohol-dependent drivers.

Trends in the alcohol-fatal crash problem in Canada

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OBJECTIVE: This paper examines recent trends in the alcohol-fatal crash problem in Canada to determine if the magnitude of the problem has been increasing or decreasing.

METHOD: Information on the presence and amount of alcohol in fatally injured drivers is used in this paper as an index of the alcohol fatal-crash problem in Canada. Trends in the problem are examined using a variety of indicators derived from the "STRID" fatality database (Strategy to Reduce Impaired Driving) – e.g. the percent of fatally injured drivers positive for alcohol.

RESULTS: Canada experienced a steady decline in the percentage of fatally injured drivers who had been drinking from 1987 to 1990. This was followed by an increase in 1991 and basically no change in 1992. Between 1992 and 1999 the percent of fatally injured

drivers with positive BACs gradually declined – i.e., 48% in 1992, compared to 33% in 1999. Since 1999, the percent of fatally injured drivers with positive BACs has increased to 38% in 2001, declined to 35% in 2002, increased to 38% in 2003, and returned to 35% in 2004.

CONCLUSIONS: The magnitude of the alcohol-fatal crash problem in Canada has fluctuated in recent years and the problem remains significant. Further efforts are needed to address it effectively.

Alcohol related road accidents in Germany – status till 2005

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In 2005, 22,004 alcohol-related injury accidents and 603 killed were registered in the official German accident database. These figures represent 6.5% of all injury accidents and 11.2% of all road accident fatalities.

Although alcohol-related accidents still contribute to a considerable amount to the overall number of injury accidents they have been declining steadily during the last decade – by more than 40% - whereas the overall number of injury accidents has declined by 13% during the same time. Moreover, the number of casualties shows a respectable downward trend. Especially the reduction in fatalities by 65% between 1995 and 2005 is far above average.

As a result the proportion of accident-involved road users influenced by alcohol decreased from 4.9% to 3.4% between 1995 and 2005 whereas the share of alcohol related casualties was reduced from 9.8% to 6.5% during the same period of time.

The presentation will give an overview of the development of alcohol-related injury accidents between 1995 and 2005 as well as an insight into their structure – with special emphasis on the topics of driver's age and BAC-levels.

Trends in impaired driving in the United States: time for a new paradigm?

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This paper is presented as part of the session on worldwide trends in impaired driving. It provides analysis of the current situation in the United States.

After 15 years of decline, in the last decade, the percentage of fatal crashes that involve alcohol has stalled at about 40%. It is apparent that policies, enforcement levels, geographic, and other factors play a role in determining the level of alcohol involvement. States vary widely in the involvement of alcohol in fatal crashes with state-to-state percentages of fatal crashes involving alcohol ranging from 12% to 50% in 2005.

The lack of progress may in part result from the fact that, while many effective strategies are well known, they are not implemented as widely or as vigorously as possible. For example, well-publicized sobriety checkpoints have been shown to have a significant impact on impaired driving, but they tend to be implemented only sporadically and only in limited areas of the country.

Some aspects of the impaired driving problem have been particularly difficult to change. For example, a higher percentage of motorcycle operators in fatal crashes have alcohol in their systems as compared to drivers of passenger cars. In other areas, some progress is apparent. The number of licensed drivers younger than 21 declined by 14 percent between 1982 and 2004 while the number of underage drinking drivers in fatal crashes declined by 62 percent during that period. Thus, it appears that the MLDA 21 law and the national zero tolerance law had a substantial effect on underage drinking and driving.

It may be that we are approaching the limits of policy and deterrence to suppress impaired driving. This possibility, along with the dramatic advances in technology, has led some advocates and policy makers to promote the wider application of technological approaches to preventing impaired driving. Currently, many states are considering more vigorous use of alcohol ignition interlocks for impaired driving offenders and two states have passed legislation mandating interlocks for first offenders. A Blue Ribbon panel has also been organized to examine how technology could be used to prevent driving over the legal limit in the general population through the installation of alcohol detection technologies in all vehicles. Such strategies would mark a new direction for the impaired driving field and could bring about the progress that has been lacking for so long.

Worldwide trends in alcohol and drug impaired driving

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This paper will summarize the latest trends in a number of industrialized countries around the world and discuss the reasons for the changes that occurred, and review current programs designed to produce further reductions in impaired driving. In the decade of the 1980s, there were impressive declines in drinking and driving in much of the industrialized world. The declines included about 50% in the Great Britain, 28% in Canada and The Netherlands, 32% in Australia, 37% in Germany and 26% in the U.S. These declines did not continue in the early part of the 1990s. In some countries, there were actually increases. Toward the middle and latter

part of the decade the increases stabilized and we again began to see some decreases. However, these decreases have been at a slower rate than the dramatic decreases in the 1980s. Toward the end of the 1990s and in the new century, the record has been mixed. Clear trends have emerged. Some countries (France and Germany) continued to reduce drinking and driving while in other countries (Australia, Canada, United Kingdom and the United States), there was stagnation and in some cases small increases or even large increase as was the case in Sweden. Trends on drug impaired driving are also beginning to emerge in some countries. These trends will also be discussed.

Drugs in driving – the South Australian experience

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On the 1st of July 2006 new Road Traffic legislation came into effect in South Australia, which allowed for the random drug testing of oral fluid from drivers. It also allowed for the testing of blood samples from drivers injured in motor vehicle accidents for drugs as well as alcohol.

The random testing of drivers for alcohol and the testing of blood from all people involved in a motor vehicle accident who attend a prescribed hospital has been in force in South Australia since the early 1960's. New legislation has now allowed the random testing of drivers and the testing of injured drivers for three prescribed drugs (Δ^9-THC), methylamphetamine and 3,4-methylenedioxymethamphetamine (MDMA).

All positive oral fluid samples from the roadside testing are submitted to the Forensic Science Centre for confirmation. This presentation will examine the number of confirmed positive samples and the proportion of each of the prescribed drugs present. This data will be compared to the proportions of these prescribed drugs found in the blood samples taken from injured drivers.

It is estimated that the South Australian Police will conduct 9,000 oral fluid tests in the 12-month period to the end of June 2007 with an expected positive rate of approximately 3.4%. It is estimated that approximately 1,000 blood samples from injured drivers will be analysed in this same period.

The methodology used for the laboratory analysis will be discussed. Solid phase extraction followed by derivatisation with PFPA and GC-MS analysis is used for the analysis of amphetamines in oral fluid and blood. Liquid-liquid extraction and derivatisation with PFPA and GC-MS analysis is used for the analysis of Δ^9-THC in oral fluid and blood.

Intoxications : case reports
Intoxications : études de cas

Postmortem cesium concentrations in a cancer patient: a case report

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CASE REPORT: Cesium (Cs) is an alkali metal found in the earth's crust. Low incidence of cancer has been observed in geographical areas where soil or water is rich in cesium. Tumor burden has also been reported to decrease in patients treated with cesium chloride (CsCl). The efficacy and safety of CsCl therapy is scientifically not well established and therefore, not used in traditional medicine. Instead, it is used as a complementary and alternative therapy for cancer. Herein, we present a case of a cancer patient self-medicating with CsCl and the postmortem concentrations of Cs in tissues.

A 55-year-old female presented with metastatic carci–noma of the gastro-esophageal junction. She was treated with chemotherapy and radiotherapy prior to laparotomy and jejunostomy at which time the diagnosis was confirmed to be advanced metastatic adenocarcinoma of probable ovarian origin. She was treated with oxycodone and IV hydromorphone to control her pain. Oxycodone was then substituted with hydrocodone and fentanyl. Fentanyl dose was gradually increased from 100 to 300 ug/hr over a three and the half month period. She also received magnesium and potassium supplements since her serum electrolytes were low. She then resorted to CsCl as a complementary and alternative therapy. The alleged dose she took was 6-9 g/day.

METHODS: Cesium was measured by ICP/MS at National Medical Services Inc. Fentanyl, hydrocodone and diphenhydramine were quantified by GC-MS post liquid/liquid extraction. Hydromorphone and hydrocodone were quantified by LC-MS following solid phase extraction.

RESULTS: Postmortem toxicological analyses of peripheral blood revealed the presence of: fentanyl (12 ng/mL), hydrocodone (13 ng/mL), hydromorphone (< 1 ng/mL), and diphenhydramine (0.50 mg/L). Cesium concentrations were: peripheral blood (1250 mg/L), central blood (670 mg/L), vitreous (112 mg/L), liver (160 mg/Kg), kidney (138 mg/Kg), and brain (155 mg/Kg). Baseline concentrations are < 0.27 mg/L in blood (National Medical Services Inc.) and 3-5 x 10-3 mg/Kg in control tissues (Centeno et al). The reported Cs concentrations in tissues from 2 decedents are: blood (84, 28 mg/L), liver (337, 40 mg/Kg), kidney (120, 44 mg/Kg), and brain (46, 17 mg/Kg)

(Centeno et al). While these results show that the highest concentrations of cesium are in the liver and kidney, our results demonstrate that cesium is highly concentrated in peripheral blood. The blood Cs levels in our case is 5-15 times higher than the reported ones.

CONCLUSIONS: Although the forensic implication of CsCl therapy in humans is currently not well understood, CsCl is potentially a life threatening agent. Experimentally, CsCl is used to induce QT interval prolongation and cardiac arrhythmias in animals. These conditions in addition to hypokalemia and syncope have been recently reported in human patients who received CsCl as an alternative or preventative therapy against cancer. The cause of death in the presented case was signed as cardiac dysrhythmia associated with probable cesium toxicity and manner of death was natural.

Toxicological analysis of atracurium besylate in biological materials by using HPLC

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AIMS: Atracurium Besylate is a highly selective, competitive (non-depolarizing) neuromuscular blocking agent used in anesthesiology. Following intravenous administration atracurium is predominantly degraded, under physiological temperature and pH, by Hoffmann elimination to laudanosine. The aim of this study is to present a method for the toxicological analysis of atracurium in biological materials using HPLC.

METHODS: An isocratic reversed phase HPLC method coupled with UV detection has been developed for the determination of atracurium in human blood, urine, bile and tissues from the injection sites. The extraction technique used was simple and rapid; where blood, urine, bile and macerated injection site tissue samples were acidified with 0.5 M sulphuric acid to pH 4.2 and vortex mixed for 1 minute with acetonitrile. The samples were centrifuged; acetonitrile layer was separated and evaporated. A 20 µL aliquot of acetonitrile extract was injected onto the HPLC column. The extraction recovery was 79.6% at 50 µg/mL atracurium in blood.

The extracts were separated with a Lichospher Si-60 (150 mm x 4.6 mm I.D, 5 µm particle size) column. A mobile phase consisting of acetonitrile and 0.1 M dipotassium hydrogen ortho phosphate buffer (pH=5) in a ratio of 40:60 v/v was used at a flow rate of 1mL/min and UV detection was done at 280 nm.

RESULTS: Linear detector responses were observed for the calibration curve standards in the range of 0.2 - 100 µg/mL. The limit of detection (S/N ratio =3) and limit of quantitation (S/N ratio=10) for atracurium were 0.1µg/mL or 2 ng on column and 0.4 µg/mL or 8 ng on column respectively. The Relative Standard Deviation for atracurium besylate determination at 50 µg/mL was 2.2%.

The selectivity of the method was verified against endogenous compounds due to the matrices for which blank blood and urine samples extracted separately in the same way was injected and checked for the absence of interfering compounds.

CASE REPORT AND CONCLUSIONS: The method was validated and successfully applied in a case of fatality, where a 31 year old Sri Lankan male anesthetist, attached to a Government hospital was found unconscious in his duty room at night. An IV canula with a syringe was found in situ in one hand and an empty atracurium besylate vial was found at the scene. Medical investigations revealed that he was already dead and a postmortem was carried out by the Judicial Medical Officer. The samples from the body including blood, bile, urine and tissues from the injection site together with the butterfly IV set with an empty injection syringe and empty atracurium vial recovered from the scene were sent to the Toxicology Section of the Government Analyst's Department for examination. On analyzing the samples, 10.5 µg/mL, 28.8 µg/mL and 51.8 µg/mL atracurium besylate were identified in blood, bile and urine samples respectively. Further, atracurium besylate was identified in the empty vial, empty injection syringe recovered from the scene and the tissues taken from the injection site. This was the first recorded fatal case of atracurium besylate poisoning in Sri Lanka and possibly the first case of suicide in Sri Lanka involving atracurium besylate.

Aniline lethality in humans: metabolic fate and pharmacokinetics in an animal model

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AIMS: A 55-year-old man was brought to the emergency room after vomiting, confusion and severe general cyanosis. Immediate toxicological analyses revealed this was the result of aniline ingestion. He died 40 hours later. An autopsy and toxicological investigations were required and an animal model was used for a better knowledge of aniline metabolism.

METHODS: Antemortem and postmortem toxicological analyses were performed in blood and stomach contents by GC-MS and HPLC-DAD. Hair specimens were

analyzed by LC-MS/MS. Methemoglobin levels were determined in blood on a CO-oximeter. To determine the kinetic profile of aniline and its metabolites and the subsequent production of methemoglobin, a large white pig was used. The animal received a single oral dose of aniline (0.07 mL/Kg) and 10 blood samples were collected during a 360 min period.

RESULTS: Human case. At admission, a methemoglobin level of 68% was determined in blood. Aniline was present in stomach contents (0.47 µg/mL) and in plasma (8 ng/mL). Acetaminophen and acetanilide were also present in plasma (5.5 and 22.5 µg/mL, respectively) and in stomach (20 and 12 µg/mL, respectively). After an immediate stomach pump treatment and methylene blue infusion (2 mL/Kg), methemoglobin decreased to 25.5% after 4 hours but then increased up to 69.0% after 18 hours. Two successive exchange transfusions were then performed and methemoglobin subsequently decreased to a level of 4.6% but the patient died from a systemic failure. Aniline and its metabolites were not identified in hair. Animal experiment. After oral aniline administration to the pig, the plasma concentrations and the associated methemoglobinemia were as follows:

Time (min)	Aniline (ng/mL)	Acetanilide (µg/mL)	Acetaminophen (µg/mL)	Methemoglobin (%)
0	0	0	0	0,5
15	3,7	26,9	1,2	0,8
30	4,5	24,4	2,7	2,2
45	4,2	27,6	4,8	3,0
60	4,2	28,6	4,9	5,2
90	4,5	30,3	4,5	5,7
120	4,1	30,5	7,6	9,1
180	3,5	28,2	9,5	11,8
240	2,5	27,1	8,0	11,7
360	1,7	30,8	10,8	11,4

CONCLUSIONS: This human case indicates that in an acute intoxication with aniline involving high levels of methemoglobin, exchange transfusion should be performed rapidly after methylene blue infusion. The results obtained in human as well as in pig indicate that when administered orally, aniline is rapidly metabolised to acetanilide and acetaminophen. Furthermore the very low concentration ratio of aniline to metabolites suggests a pre-systemic metabolism. The low concentration for aniline in plasma in contrast to stomach contents also supports this hypothesis. The animal experiment indicated that both metabolites were still present at increasing concentrations 6 hours after aniline administration. Aniline concentration decreased after 90 min whereas methemoglobin levels were still increasing at the end of the experiment.

Getting high by smoking hyoscine butylbromide?!

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AIMS: Hyoscine butylbromide (N-butyl-S-(-)-scopolammonium bromide), commercialised in Europe, under the brand name "BUSCOPAN®", is commonly used as antispasmodic agent. Recently our laboratory was requested to elucidate the case of a prison inmate claiming to smoke crushed BUSCOPAN® tablets in cigarettes and experiencing agitation, hallucinations and aggressive behaviour. The aim of this study is to determine if thermal degradation of hyoscine butylbromide in smoked cigarettes generates hyoscine (S-(-)-scopolamine)?

METHODS: A series of 8 cigarettes with and without filters, fortified with 10 mg of hyoscine butylbromide, were investigated by different smoking modes (continuous, puff-by-puff). The smoke of the burning cigarettes was collected with an ammonium buffer – acetonitrile mixture, whereas the ashes and the filter were extracted with methanol. After centrifugation and addition of atropine (I.S.) the samples were analyzed by LC-MS/MS in ESI mode. Validation experiments included specificity, linearity, sensitivity, precision and accuracy (*Method published in: J. Anal. Toxicol., 31 (2007), 220-223*). In particular, the potential breakdown of hyoscine butylbromide during analysis was excluded as no scopolamine could be detected when hyoscine butylbromide alone was injected into LC-MS/MS.

RESULTS: Hyoscine was detected in the 3 matrices (smoke, ashes, and filter) in all smoking modes. Quantities of hyoscine in smoke were highest in cigarettes without filters (range 131 – 238 µg/cigarette) and lowest in cigarettes with filters but smoked in the puff-by-puff mode (range 98 – 166 µg /cigarette). The results indicate that a consumer smoking a cigarette fortified with a crushed BUSCOPAN® tablet containing 10 mg of hyoscine butylbromide inhales 100 to 150 µg of hyoscine. Although literature reports about hyoscine inhalation is lacking, pulmonary administered drugs are generally rapidly and nearly completely absorbed. Thus even low doses of hyoscine may cause the effects observed in the case of the prison inmate.

CONCLUSIONS: Reports of persons claiming to smoke Buscopan® should be taken seriously and prescription of Buscopan® tablets to prison inmates should be reconsidered. Further studies will be necessary to elucidate the thermolysis reaction mechanism.

An accidental intoxication with *Veratrum album*

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CASE REPORT: Veratrum species contain a range of alkaloids that induce bradycardia and hypotension. These alkaloids act by increasing the permeability of the sodium channels of excitable cells, causing them to fire shortly and then leaving them refractory. Early symptoms after ingestion of *Veratrum* plant extracts, such as gentian spirit, consist of vomiting, nausea and abdominal pain. They are followed by severe bradycardia and hypotension.

A 49-year-old man reported an ingestion of two glasses (approx. 2 x 20 mL) of an alcoholic extract supposedly containing yellow gentian (*Gentiana lutea*). Shortly, after ingestion he developed nausea, vomiting and oral paraesthesia. On admission to the hospital he suffered from severe bradycardia (35 min^{-1}) and hypotension (50/30 mm Hg). For primary detoxification he received activated charcoal. Further medications were metoclopramide, ondansetron, atropine and volume. With suspicion of an intoxication with *Veratrum* alkaloids, the spirit and a serum sample were sent to our institute for investigation.

METHODS: The identification and quantitation of five *Veratrum album* alkaloids (protoveratrine A (ProA) and B (ProB), veraridine, cevadine and jervine) is based on a liquid-liquid-extraction followed by LC-ESI-MS/MS. Analytical separation was carried out using a Varian Pursuit 5 PFP column (150 x 3.0 mm). The gradient consisted of a mixture of solvent A (methanol: 0.1% HAc with 10 mM NH_4Ac (97:3) and solvent B (0.1 % HAc with 5 mM NH_4Ac:methanol (90:10)). The flow rate was 0.55 mL/min, the oven temperature was 60oC and the analysis time was 6 min. For serum, quantification was performed in the MRM mode using a 5-point calibration (100, 200, 500, 1000 and 1500 ng/L) curve. The LLOQ is 10 µg/L (S/N > 10, extract) and 100 ng/L (S/N > 10, serum). The quantitation of ethanol was carried out using a headspace-GC.

RESULTS: Analytical results demonstrated intoxication with alkaloids from *Veratrum album*. In the spirit the ProA concentration was 20.4 mg/L and ProB 13.7 mg/L, respectively. The yellow-coloured spirit contained 25% ethanol, thus indicating, that the extract was homemade. The calibration curves of all compounds were linear ($R \geq 0.995$) and the intraday-assay precision CVs (n=2) were < 10% (300 ng/L) and < 14% (800 ng/L), respectively. The serum concentration of ProA was 1160 ng/L and of ProB was 402 ng/L. Veratridine, cevadine and jervine were not detected in either the extract or serum sample. After treatment, the patient completely recovered from symptoms within 24 hours and left the hospital.

CONCLUSIONS: A man ingested a homemade spirit containing *Veratrum album*. We report the first quantitative analysis in serum of its components confirming his intoxication with ProA and ProB. After treatment, the patient was discharged from the hospital within 24 hours.

Lysergic acid monoamide through the internet: the case of *Ipomea violacea*

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AIMS: Researchers working around the 1960s were interested in the psychedelic properties of substances used by young people, such as LSD, mescaline and psilocybin. Current scientific literature reveals little new information, and the most important sources of knowledge on the nature of these active principles thus come from research carried out 40 years ago. Although many "smart drugs", with uncertain psychoactive properties, are marketed today in the form of seeds to be cultivated and harvested, perfume exhalers for home use, incense sticks and pastes, etc., it is legitimate to suspect that their true use is different from that declared on the label. The ease with which they can be obtained through the internet has aroused new interest in them. The aim of this work was to confirm the presence of lysergic acid monoamide (LSA) in seeds, acquired through the internet, of a plant belonging to the *Convolvulaceae* family, *Ipomea violacea*. LSA is a natural hallucinogen known for its psychotropic effects such as: psychotomimetic (alterations in thinking, perceptions, and state of consciousness), visual (bright colours associated with a sensation of serenity) and dysphoria, which may lead to a hypnotic effect.

METHODS: Five mg of ground seeds, to which 1 µL of a 1 mg/mL solution of LSD (SIGMA standard) had been added, were extracted in a mixture of organic solvents (methanol/chloroform 1:1 v/v) in an ultrasound water bath for 24 hours at 60°C. In parallel, SPE was used to extract a 5 mg aqueous infusion of *Ipomea violacea* seeds to which the internal standard had been added. Both extracts were analysed by GC/MS (Varian, Saturn). In both cases, LSA was detected, but the aqueous extract had a higher concentration than that obtained with organic solvents. Quantitation was performed using LSD as a standard. LSA only differs from LSD in having $-NH_2$ in place of the $-N(C_2H_5)2$ of LSD. LSA and LSD have molecular ions at m/z 267 and 323, respectively, in addition to the typical fragmentation ions (m/z 221 and 207), common to all ergot alkaloids. In the light of these

results, it was possible to use the fragmented ion of mass m/z 221 to quantify the active principle by means of a calibration curve (LSD standard, range 0.01-0.1 mg/L). In order to verify the stability of LSA, the extracts were stored in the dark at 7°C, and analysed at 6, 30, 36 and 50 hours after their preparation. Samples of extracted seeds and of the LSD standard were then left for 7 days at room temperature and exposed to daytime light, after which they were again analysed by GC-MS.

RESULTS: The LSA concentration in seeds was 0.023 mg/mL. At 50 hours after preparation the concentration of LSA was 60% of the initial concentration. After 7 days, the colour of the solution had changed from neutral to bluish-violet, and the active principle could no longer be detected.

CONCLUSIONS: The ease with which *Ipomea violacea* seeds can be obtained through the internet should arouse greater interest on the part of forensic toxicologists. Extraction by SPE also highlights the fact that LSA is soluble in water, confirming the possibility of extracting it by infusion.
As *Ipomea violacea* belongs to the family of ergot alkaloids, in cases of suspected use/abuse of the plant, toxicological analyses must be carried out rapidly, applying analytical methods for detecting LSA.

Biological monitoring of phenoxyethanol in occupational exposure by analysing urinary phenoxyacetic acid

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AIMS: Phenoxyethanol (PE) is a glycol ether used as preservative in cosmetic products that can easily be absorbed through the skin. It is eliminated in urine as phenoxyacetic acid (PEAA), a metabolite that can be used in biological monitoring for occupational exposure. Used as an anaesthetic during transfer of fish between basins or during handling for obtaining measurements, dermal exposure to PE may occur in workers not wearing gloves. The aim of this study was to evaluate workers for exposure to PE through the measurement of PEAA concentrations in urine.

METHODS: Urine samples were collected for eleven workers exposed to PE at the start of the week (reference values) and at pre-shift and post-shift at the end of the week. Urine samples (100 µL) with internal standard (3-chloropropionic acid) were extracted with dichloromethane and derivatised with anhydrous pentafluoropropionic acid for 16 h. Chromatographic separation is performed on a BPX5MS column (SGE) and detection is performed by GC/MS-NCI using methane. Acquisition is performed in SIM with a quantitation of PEAA was performed using the 151 m/z ion.

RESULTS: Urinary PEAA concentrations of workers at the end of the week ranged between 0.11 and 4.69 mg/g creatinine pre-shift (mean = 1.82 +/- 1.34 mg/g creatinine, n = 11 workers) and between 0.18 and 85.71 mg/g creatinine post-shift (mean = 22.70 +/- 22.71 mg/g creatinine, n = 10 workers). At the start of the week, reference values ranged between 0.12 and 2.72 mg/g creatinine (mean = 0.79 +/- 0.77 mg/g creatinine, n = 10 workers).

CONCLUSIONS: No biological exposure index for phenoxyacetic acid in occupational exposure has been defined. This study shows that the monitoring of PEAA is a necessary tool for estimation of workers exposure to PE in pisciculture. Variations of urinary concentrations post-shift can be explained by length of exposure for the eleven workers. It seems important to evaluate exposure of workers after transferring of fish between basins and after manipulation to obtain measurements in order to identify which subjects are more exposed and then adapt specific preventive actions.

Development and application of an HPLC-FL method for the determination of n-substituted piperazines

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AIMS: Since N-benzylpiperazine (BZP) has been regulated as a narcotic since 2003, sensitive determination of *N*-substituted piperazines such as BZP and its analogues is required in Japan. In this study, an HPLC-fluorescence detection method for the determination of BZP and its analogues in rat plasma is described.

METHODS: Fluorescence labeling of BZP, *p*-methoxyphenylpiperazine (*p*-MeOPP) and *o*-methoxyphenylpiperazine (*o*-MeOPP) was performed at 60ºC for 30 min with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl) under the alkaline conditions. *m*-Methoxyphenyl-piperazine was used as an internal standard. The analytes were isolated using solid-phase extraction (BondElut®C18). The DIB-piperazine derivatives were separated on a Daisopak-120-5-ODS column (250 x 4.6 mm) with 0.1 M acetate buffer (pH 3.5) : CH_3CN : MeOH (40:45:15, v/v/v) at a flow rate of 1.0 mL/min, and monitored at 340 nm (Ex) and 445 nm (Em).

RESULTS: The DIB derivatives could be well-separated from the interfering peaks of plasma. The retention times for DIB derivatives of BZP, p-MeOPP and o-MeOPP were 15, 31, 35 min, respectively. Calibration curves of piperazine derivatives showed

good linearities with a correlation coefficient of greater 0.997. The detection limits for BZP, *p*-MeOPP and *o*-MeOPP at a signal-to-noise ratio of 3 were 0.57, 47.7 and 5.1 ng/mL, respectively. The intra- and inter-day precision studies demonstrated CV's of less 4.3% (n = 5). Furthermore, BZP monitoring in rat plasma after a single administration of BZP (2 mg/Kg, *i.p.*) was performed as an application study of the proposed method. The BZP concentrationss in rat plasma ranged from 2 – 680 ng/mL up to 360 min after administration.

CONCLUSIONS: The proposed HPLC-fluorescence detection method was validated for the determination of piperazine derivatives in rat plasma, and its applicability could be successfully confirmed.

Marijuana and driver impairment
Cannabis et altérations du comportement du conducteur

Cannabis intoxication and fatal road crashes in France: population based case-control study - results and comparison with the alcohol

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OBJECTIVE: To evaluate the relative risk of being responsible for a fatal crash while driving under the influence of cannabis and/or alcohol and the fraction of fatal crash related deaths attributable to cannabis and alcohol.

METHODS: Population based case-control study. 10,748 drivers involved in fatal crashes in France from October 2001 to September 2003 are included in the study. The blood alcohol and cannabis (Δ^9-tetrahydrocannabinol concentration) are known.

The cases (6,766) are drivers responsible for the fatal crashes. The controls (3,006) have been selected from drivers not at fault. Cannabis and alcohol prevalence is standardised on drivers not at fault who are involved in crashes resulting in slight injuries.

RESULTS: After adjustment for different factors, the relative risk of being responsible for a fatal crash while driving under the influence of cannabis alone (THC > 0) is 1.8, 8.5 with alcohol alone (BAC > 0) and 14.0 when both cannabis and alcohol are positive. A significant dose effect is found for cannabis like for alcohol.

The adjusted fraction of fatal crashes attributable to cannabis is 2.5% and 28.6% for alcohol. In addition to these numbers linked to the responsibility of drivers under influence, an estimation of fatal crashes attributable to the greater vulnerability of drivers under influence of cannabis (1.5%) and alcohol (11.0%) are estimated. The prevalence of cannabis (2.9%) estimated for the driving population in France is lower than that for alcohol (5.3%) but higher (2.7%) than for alcohol concentration over legal threshold (≥ 0.5 g/L).

Drivers under influence of cannabis are mainly young men. Half the fatalities for at fault crashes involving drivers who tested positive for cannabis are younger than 25; they represent 17.9% of the total of dead people under 25 on crashes. Alcohol related fatal crashes affect older people nevertheless, because of its high levels of prevalence and risk, there are more deaths of young people (< 25 years) related to crashes with drivers at fault positive to alcohol (38.5%).

Fatal crashes with drivers under influence of cannabis or, for the most part, alcohol are very frequent at the night, especially on Saturday. On Saturday night, they are responsible for 3 fatal crashes out of 4.

CONCLUSIONS: Driving under influence of cannabis increases the risk of being responsible of a fatal crash. However, this risk and its share in fatal crashes are much lower than those, well known, associated with alcohol.

Relationship between THC-concentration in blood and impairment in apprehended drivers

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BACKGROUND: The most important psychoactive ingredient in cannabis, Δ^9-tetrahydrocannabinol (THC) is one of the most frequently detected substances in blood samples from suspected impaired drivers in Norway. There is growing concern over possible links between the use of cannabis and increased risk of motor vehicle accidents. Experimental studies have provided useful information on the role of THC and dose-effect relations with respect to psychomotor performance. The main purpose of the present study was to investigate whether a physician's judgment on impairment in a real life setting among suspected drugged drivers, was related to blood THC-concentration.

METHODS: In Norway a police physician performs a clinical test for impairment (CTI) shortly after apprehension. The Norwegian Institute of Public Health analyzes blood samples from all drivers suspected of driving under the influence of non-alcoholic drugs. In the present study 589 samples from approximately 30,000 cases of suspected drug impaired driving from the period 1997-99, contained THC as only drug. In 456 of these cases a conclusion of the CTI was available.

RESULTS: 230 (54%) drivers were considered not impaired and 226 (46%) impaired. Impaired drivers had higher blood THC concentration than the drivers who

were judged as not impaired (median; 2.5 ng/mL (range; 0.3 - 45.3 ng/mL) vs 1.9 ng/mL (range; 0.32 - 24.8 ng/mL), ($p < 0.05$). Furthermore, drivers with blood THC concentrations above 3 ng/mL had an increased risk for being judged impaired compared to drivers with lower concentration ranges.

CONCLUSIONS: The relationship between the concentration of THC in blood and risk of being assessed impaired found in this cross-sectional study of suspected drugged drivers, supports findings from previous experimental studies of concentration related effects of THC on psychomotor performance and driving skills.

Cannabis effects on cognition and psychomotor function in daily Cannabis users

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It has previously been demonstrated that the cognitive and psychomotor effects of cannabis are related to THC concentrations in serum and oral fluid. It is however unclear whether previous experience with cannabis is a mediating factor in the impairing effect of cannabis.

The aim of the present study was to investigate whether occasional cannabis users demonstrate the same cannabis induced impairment as experienced cannabis users. Therefore we compared the effect of a high dose of cannabis on cognitive and driving related tasks in occasional and daily cannabis users.

Twelve daily cannabis users (> 6 times/week) and 12 occasional cannabis users (< once/week) participated in a mixed, placebo controlled, cross-over study. Treatment consisted of placebo and a single dose of 500 µg/kg THC. Performance tests were repeated four times up to 8 hours after smoking, and included measures of motor control, divided attention, executive functioning, motor impulsivity, change blindness and memory. Physiological measures and blood and saliva samples were taken at baseline and at regular intervals up to 8 hours post-smoking.

On most performance tests, occasional cannabis users appeared to be significantly more impaired after smoking cannabis than daily users. However, daily users had more difficulties inhibiting their response in the motor impulsivity test. In the daily users, concentrations of THC and its metabolites 11OH-THC and THC-COOH, showed higher peak levels in serum.

Although daily cannabis users had higher concentrations of THC and metabolites of THC, the occasional users experienced more impairment after smoking the cannabis cigarette. These data suggest that daily users have developed some kind of tolerance for the psychopharmacological effects of high doses of cannabis.

Simulated driving performance of inexperienced and experienced drivers after single doses of cannabis, alcohol and their combination

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Previous research suggests that while drivers may be able to maintain basic levels of driving skill (with the exception of increased SDLP) after using cannabis, they may not be equally able to perform in situations of higher mental load. Certain populations, such as young novice drivers, may be particularly susceptible to the effects of these drugs on driving. This study aimed to establish the effects of cannabis and alcohol, alone and in combination, on the performance of drivers who are required to perform a secondary task while driving, and who are placed in a range of safety-critical scenarios.

Inexperienced (18-21 yrs, licensed for less than 2 yrs) and experienced drivers (25-40 yrs with at least 7 yrs driving experience), with a history of some alcohol and cannabis use, were administered three doses of alcohol (placebo, 0.35 and 0.65 mL/Kg) and three doses of cannabis (placebo, 16.5 mg and 33 mg Δ^9-THC), alone and in combination, in counterbalanced order in nine separate sessions. Alcohol (breathalyser) and blood THC levels were measured during each session. Participants drove a simulator through a variety of driving scenarios, performed a battery of field sobriety tests, and completed a memory and mood task. The driving task included residential, rural, arterial, and freeway scenarios on the mid-range simulator in which the workload demands of the driving task were manipulated. These drives included a secondary task, and headway maintenance and selection tasks. In a separate risk perception drive participants were exposed to unexpected and risky situations (e.g., a pedestrian jumping out from behind a parked car) that required a rapid decision and response.

While the arterial drive involved higher workload, the residential, rural, and freeway drives were low workload drives and represent driving in normal conditions. This study found that even very low doses of cannabis, in

normal low-demand driving environments, caused impairment in driving ability. The impairment in these normal low workload environments was evident as increased variability in driving performance. Further, high levels of cannabis generally induced greater impairment than lower levels. The impairment observed following cannabis use was magnified in situations involving more complex driving environments and reactions to events occurring outside the vehicle. Driver experience affected performance when workload was increased through the addition of secondary tasks while driving. In situations of high workload and high cannabis, experienced drivers appeared to compensate by performing the secondary task more slowly. Alcohol, at the doses employed in this study which were under 0.05%, had few effects and did not add to the effects of cannabis. These data lend support to the continuation of roadside drug testing for cannabis.

Assessment of driving capability through the use of clinical and psychomotor tests in relation to blood cannabinoids levels following oral administration of 20 mg dronabinol or of a Cannabis decoction made with 20 or 60 mg Δ^9-THC

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THC (Δ^9-Tetrahydrocannabinol) is the most frequently detected drug in blood of drivers suspected of driving under the influence of drugs. In experimental studies using driving simulators and on-the-road driving tests, cannabis impairs cognition, psychomotor function, and actual driving performances. However, the simultaneous measurement of blood cannabinoids concentrations, of psychomotor performances, and of driving capability, especially after oral ingestion, has rarely been determined. Furthermore, most of these studies have been performed with low to medium doses of THC. The present study used a double-blind crossover design to compare the effects of medium (16.5 mg THC) and high doses (45.7 mg THC) of hemp milk decoctions, of a medium dose of dronabinol (20 mg synthetic THC, Marinol®) and of a placebo on several skills required for driving. Objective signs such as conjunctive reddening, pulse rate and arterial pressure were also recorded. Time concentration-profiles of THC, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) in whole blood were determined by gas chromatography-mass spectrometry- negative ion chemical ionization. Compared to smoking studies, relatively low concentrations were measured in blood. The highest mean THC concentration (8.4 ng/mL) was achieved 1 hour after ingestion of the highest dose of THC (hemp milk decoction). Moreover, individual blood levels showed important intersubject variability.

Eight male subjects aged 22 to 30 years, all occasional cannabis smokers, were enrolled. Two subjects were withdrawn from the study after administration of dronabinol or hemp milk decoction containing the medium dose of THC because of development of transient psychotic symptoms (depersonalization, paranoid feelings and derealisation). The willingness to drive was influenced by the importance of the requested task. For example, under significant cannabinoids influence, the participants refused to drive when they were asked whether they would agree to accomplish several unimportant tasks (e.g., driving a friend to a party). Most of the participants reported a significant feeling of intoxication and did not appreciate the effects, notably those felt after dinking the strongest decoction. Nausea was often reported and vomiting was also observed. These adverse effects are aggravating factor for several subjects. A slight to moderate conjunctive reddening was consistently observed, which was more intense after the highest dose of THC. Road sign and tracking testing revealed obvious and statistically significant differences between placebo and treatments. A marked impairment was observed after ingestion of high dose of THC (hemp milk decoction).

This controlled clinical study points out the negative influence on fitness to drive after medium or high dose oral THC. Moreover, this study shows that although large doses of THC were ingested and obvious psychoactive effects observed and performance impairments monitored, whole blood levels of THC remained lower than 13 ng/mL.

If moderate and heavy Cannabis users smoke a joint – comparative pharmacokinetics and graphing performance data after smoking 500 mcg THC/kg b.w. vs. placebo

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OBJECTIVES: The dosage – serum concentration – effect – relationship in cannabis users with reference

to their driving ability is still in discussion. In a first study, cognition and motor control in moderate cannabis users after smoking of THC in 2 doses and placebo, respectively, were investigated and correlated with pharmacokinetic data (Ramaekers et al., DAD, 2006). It is concluded that serum THC concentrations between 2 - 5 ng/mL establish the lower and upper range of a legal THC limit. In a follow up study we investigated a group of heavy users in comparison to moderate smokers.

METHODS: 12 regular users (THC and metabolites in serum positive before smoking) and 12 moderate users (negative in serum before smoking) were tested by CTT (Critical tracking task), TOL (Tower of London), SST (stop Signal Task) and by a novel graph-drawing-task (GDT) recorded by a computer interfaced Macomtm graphic board, on which a course had to be followed by an online pencil recording speed, pressure and lateral amplitudes (AUC) from an ideal line. Tests were repeated more times after end of smoking.

Blood sampling for analyses of THC, OH-THC and THCA in serum by GC-MSD took place before smoking (0 h) and 0.08; 0.25; 0.75; 1.00; 1.5; 2.0; 3.0; 4.0; 5.0; 6.0; 7.0; 8.0 hours after smoking. GDT was performed before and 0.25; 0.75; 4.0, 6.0, 8.0 hours after smoking.

Results in brief:

Pharmacokinetics: Moderate smokers:

Mean±SD	Cmax	tmax	C (0 h)	C (6 h)	C (8 h)
THC	49.1±24.9	0.1±0.0	n.d.	1.8±0.9	n.d.
THCOH	6.7±5.1	0.1±0.1	n.d	1.2±0.2	n.d
THCCOOH	29.0±12.3	0.3±0.2	n.d	11.0±3.8	9.2±3.9

Pharmacokinetics: Heavy smokers :

Mean±SD	Cmax	tmax	C (0 h)	C (6 h)	C (8 h)
THC	120.9±78.1	0.1±0.0	4.1±3.4	6.6±7.1	4.2±2.7
THCOH	12.3±10.8	0.1±0.1	2.5±1.8	3.3±2.7	2.4±1.3
THCCOOH	120.5±100.4	0.3±0.2	71.0±79.0	63.2±77.7	62.4±75.7

GRAPH DRAWING TASK: Moderate smokers produce higher AUC values than heavy smokers suggesting to be more impaired. Within the groups high AUC correlates with high THC concentrations.

CONCLUSIONS: Heavy smokers produced significantly higher THC Cmax than moderate smokers in spite of the same THC loading dose and also with respect of the residual C (0h) values. Moderate smokers exhibit stronger impairments in motoric performance than heavy smokers.

Validation of a model for estimating time of last Cannabis use from known concentrations of tetrahydrocannabinol and the major metabolite

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BACKGROUND: The incidence of driving while affected by cannabis is rising in parallel with increased cannabis use in the community. As the impairing effect of cannabis on driving is better understood, knowing the time cannabis was last used becomes important for determining impairment in accident investigations and clinical evaluations. Two models for predicting time of last cannabis use from single plasma cannabinoid concentrations—model I, using Δ^9-tetrahydrocannabinol (THC), and model II, using the concentration ratio of 11-nor-9-carboxy-THC (THCCOOH) to THC—were developed and validated from controlled drug administration studies by Huestis et al in 1992 and re-evaluated in 2005. The current study seeks to extend that validation by use of a large number of plasma samples collected after administration of single doses of THC to subjects in driving impairment studies and to examine the effectiveness of the models to predict time elapsed since administration of THC.

METHODS: The aggregated data of experiments involved administration of THC with and without alcohol. One data set comes from forty cannabis users who each smoked a cigarette containing either 1.74% THC or 2.93% THC. Blood samples were drawn at 25 minute intervals and THC was measured using gas chromatography-mass spectrometry. Allowing for missing data, 214 THC/time pairs were available for analysis. No measurement was made of THC-COOH. The second data set comes from another project in which subjects smoked cigarettes containing 0% THC, 1.8% THC or 3% THC with low dose alcohol (.03% BAC) or cannabis (0% THC, 1.8% THC and 3% THC) with high dose alcohol (.05% BAC). Each part was made up of six randomized, double-blind sessions. Blood was drawn at 20 minutes and 60 minutes for both THC and THC-COOH. Allowing for missing data and the placebo condition 814 data points were available. Predicted times of cannabis smoking, based on the Huestis models, were compared with actual smoking times.

RESULTS: The results validate the Huestis model for predicting time of last use of cannabis use, especially when both THC and THC-COOH levels are known.

Unlicensed drivers issues and solutions

Conducteurs sans permis problèmes et solutions

Unlicensed driving worldwide – the scope of the problem and countermeasures

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The problem of unlicensed driving is a major road safety problem in countries around the world. A survey in the U.S. showed that 20 percent - one in every five - of all fatal crashes in the U.S. involve drivers who should never have been on the road in the first place due to the fact they were unlicensed. Estimates indicate that of the 56,688 drivers in fatal crashes in the U.S. in 1998, 23.7 percent were driving without a valid license. Of drivers considered to be at fault in crashes, the percentage increased to 35.4. If all unlicensed drivers stayed off the road, there would have been 13,435 fewer drivers in fatal crashes that year in the U.S. A study in Queensland, Australia showed that unlicensed drivers represented 7.9% of drivers involved in fatal crashes. The study results suggest that unlicensed drivers are more likely to engage in higher risk behaviors than licensed drivers. A 2006 study, conducted for MADD Canada, found that suspended drivers in Saskatchewan continued to drive with no license and that these drivers were at higher risk to be in an automobile crash. In Ontario, one in fourteen fatal crashes involved an unlicensed driver.

European countries have also found that unlicensed drivers pose a higher risk. For example, in the U.K., breath test results showed that 17% of disqualified drivers who were breath tested after an accident were positive compared with the national average for all post-crash tests of 3%. A 2004 U.K. Department for Transport report found that there are around a million unlicensed drivers on U.K. roads. The report notes that while unlicensed drivers account for less than one percent of total hours driven, unlicensed drivers are up to nine times more likely to have an accident than licensed drivers. Data also indicates that the problem is increasing in France and Belgium.

This paper will discuss the worldwide scope of the problem and potential solutions, including the impoundment of the vehicles of those who are driving illegally.

Fatal and injury crashes among unlicensed drivers in Ontario, Canada

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MADD Canada and Transport Canada retained Synectics Transportation Consultants Inc. (Synectics) to conduct a study into fatal and injury crashes among unlicensed drivers in the province of Ontario during the years 1996 through 2003. The purpose of the study was to:

- Determine the fatal and injury crash involvement of unlicensed drivers;
- Determine the reason why the driver did not have a valid license at the time of the crash;
- Determine the outcome of the crash (fatal or injury);
- Determine whether or not the unlicensed driver was more likely to be at-fault in the crash based on driver action; and
- Determine whether or not the unlicensed driver was more likely to have consumed alcohol or be impaired at the time of the crash.

Based on a review of data provided by the Ministry of Transportation in Ontario, it was determined that approximately 2,000 fatal and injury crashes occur each year involving unlicensed drivers. Crashes involving unlicensed drivers were compared to overall provincial trends contained within the Ontario Road Safety Annual Reports. It was found that crashes involving unlicensed drivers were more likely to have a fatal outcome rather than an injury outcome. Unlicensed drivers were more likely to be at fault in the crash (cited as not driving properly). Alcohol or drugs were also more likely to have been reported at the time of the crash. The same analysis was also undertaken examining unlicensed drivers grouped by Criminal Code offences (alcohol related/ not alcohol related), administrative/Highway Traffic Act suspensions, and those who were never licensed or had an expired license. All groups (separately and collectively) had a significantly elevated risk ($p = 0.05$).

Unlicensed drivers were also shown to have a significantly higher likelihood of attempting to flee the scene of the crash ($p = 0.05$). Given that approximately 2,500 – 3,000 fatal and injury crashes occur each year in which the driver successfully fled the scene of the crash, it was estimated that the actual number of unlicensed drivers involved in a fatal and injury crash is 18 percent higher than that reported.

Indigenous drink driving and licensing: understanding the big picture and strategies for change in western Australia

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Indigenous people in Western Australia are highly over represented in road trauma statistics. They are also over-represented in drink driving convictions, which increases with the number and seriousness of drink driving offences. The level of imprisonment of Indigenous people for drink driving is 25 times that of non-indigenous people and indigenous people are much more likely to drive unlicensed or to have never held a driver's licence.

Responding to this difficult problem is a challenge. Aboriginal people in Western Australia, particularly in regional and remote areas, continue to experience a high degree of social, economic and cultural disadvantage and face a range of difficulties in participating in a number of systems, including licensing and drink driving programs. Such initiatives have, in the main, been developed for English speaking 'middle class' urban populations.

The Indigenous Drink Driving and Licensing Project (IDDL) was established in September 2006 to examine the extent and nature of drink driving and unlicensed driving amongst Aboriginal people in Western Australia and to make recommendation for evidence based measures to reduce the level of drink driving and unlicensed driving amongst Aboriginal people. Specifically the project aims to introduce a series of practical interventions related to primary, secondary and tertiary prevention specifically for Aboriginal communities.

The development of the strategy acknowledges the importance of creating ways to respond to the specific dynamics of contemporary Aboriginal communities, so as to reflect the cultural complexities and rather than using generalist models, its development was guided by the following principles:

- Action must align with the aspirations of Aboriginal people for their community's wellbeing and safety and involve Aboriginal people in all phases of the strategy's development;
- Full and equal participation by Aboriginal people must be achieved in the licensing system through applying principles of equity and access;
- Partnerships between government, the corporate sector, non-government organisations and Aboriginal communities should be encouraged;
- Observation of successful practice in other jurisdictions (not limited to road safety), that considers the unique needs of remote communities which this strategy serves should be incorporated; and
- Initiatives must be appropriately resourced and sustainable.

This paper will provide an overview of the Indigenous Drink Driving and Licensing program in Western Australia including detailed information on the range of measures to be roll out across the state between 2007 and 2009 to reduce the level of drink driving and unlicensed driving amongst Aboriginal people. It will be of interest to researchers, policy makers and those working with Aboriginal people in the area of road safety.

The general and specific deterrent effects of short-term license suspension

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The purpose of the current study was to evaluate the deterrent effects of short-term license suspension implemented in Saskatchewan in August 1996. Short-term suspensions in Saskatchewan can be issued to persons driving with a BAC that exceeds 0.04% but is lower than 0.08%.

General deterrence was assessed using a variety of dependent measures, including driver fatalities with various ranges of BAC and drivers involved in alcohol-related injury crashes. The analyses involved an examination of pre-post changes in the number of fatalities and injuries for the time periods of August 1991 to July 1996 and August 1996 to July 2001, using Alberta as the comparison province. Time series intervention analyses were also performed to help isolate changes that could be attributed to the introduction of the new measure.

The examination of data failed to provide compelling evidence of a general deterrent impact. The fatality data show that driver fatalities with BACs .08% or less and, in particular, those with BACs between .04% and .08% decreased in the years following the introduction of the new law in Saskatchewan and these decreases exceeded those in Alberta. However, the numbers are very small and the observed decreases were not statistically significant when compared to the comparable data from Alberta. Time series analyses also found that there was a significant downward trend in the driver fatality series that began prior to the introduction of the short-term suspension law and continued afterward. Hence, any impact of the short-term suspension law was most likely small and could not be isolated from the existing downward trend.

Specific deterrence was examined through an analysis of the incidence of re-offences among drivers who were issued short-term suspensions. Of Saskatchewan drivers who received a short-term suspension during the first two years of the law, three of every four of those who had not been convicted previously of impaired driving remained free of drinking and driving offences during the 5+ year follow-up period. This suggests that there may have been some specific deterrence for these drivers. Additionally, their rate of subsequent *Criminal Code* impaired driving offences was lower than that of Saskatchewan drivers who received a *Criminal Code* impaired driving charge.

Of those who had been convicted of impaired driving previously and received a short-term suspension during the first two years of the short-term suspension law, 100% received a subsequent short-term suspension

and/or impaired driving charge during the follow-up period. This suggests that short-term suspensions have no specific deterrence impact for drivers with prior impaired driving convictions.

DWI offenders' failure to reinstate driver's licenses

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A substantial proportion of offenders whose driver's licenses have been suspended for driving while impaired (DWI) do not reinstate their licenses when eligible to do so. Because suspended DWI offenders appear to drive less frequently and more carefully, thus reducing their risk of recidivism, it is questionable whether reinstatement should be encouraged because relicensing allows offenders to drive more and possibly with less care, which could result in a higher recidivism rate. The driving records of more than 3 million DWI offenders collected over a 7- to 10-year period in seven large states (Florida, Illinois, Indiana, Iowa, Michigan, Minnesota, and North Carolina) with more than 40 million drivers were analyzed to determine the proportion of offenders who delayed license reinstatement and the extent to which delay was associated with recidivism level. The results indicated that half of the offenders delayed reinstatement beyond 12 months. Offenders who would ultimately delay reinstatement had higher recidivism rates during the period they were suspended. Multiple offenders and those receiving longer suspension were more likely to delay reinstatement. The recidivism rates of DWI offenders who remained suspended declined over time; in contrast, recidivism levels of reinstated offenders remained elevated at 4 percent, approximately four times that of the average driver over a period of 7 years following reinstatement. The downward trend in recidivism for suspended offenders is interpreted as a result of a reduction in the driving by offenders with long-term suspensions. That group contains those for whom there is no record of reinstatement who may no longer be driving. Recommendations for policies to deal with those who delay reinstatement are offered.

Clinical and laboratory criteria for diagnosing alcohol abuse in driving license regranting procedures

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BACKGROUND: Administrative procedures for regranting driving licenses to drunk-driving traffic offenders entail a medical evaluation in Italy. In this setting, it is crucial to distinguish between alcohol use and abuse, and biochemical markers of alcohol intake alone are inadequate.

OBJECTIVE: A comprehensive method for assessing drivers by integrating clinical and biochemical diagnostic criteria is presented, the effectiveness of which was assessed in terms of the sensitivity and specificity of a set of clinical laboratory markers (singly or in combination) in diagnosing alcohol abuse and unfitness to drive.

MATERIALS: 835 drunk-driving traffic offenders (792 males and 43 females, 25-35 years old) whose licenses had been suspended were unrolled in a driving regranting protocol. At the time of their offence, 43.95% had blood alcohol concentrations (BAC) ranging from 0.5 to 1.5 g/L; 33.65% from 1.5 to 2.5 g/L; and 8.27% > 2.5 g/L, while 14.13% refused to submit to a blood alcohol test.

METHODS: The protocol involves: obtaining informed consent; examining circumstantial and medical documents; a clinical examination including CAGE and AUDIT tests to reveal any medical markers of alcohol or illicit drug abuse; collecting 1 blood and 4 urine samples over 45 days (under camera control); blood (BAC, MCV, GGT, AST, ALT), serum (CDT) and urine analyses (alcohol, ethyl glucuronide [EtG] and ethyl sulfate [EtS], drugs of abuse and psychoactive substance concentrations); final epicrisis. Statistical analysis was performed using Fisher's exact test and the X^2 test applied to 2 x 2 frequency tables. Regression models were used to identify risk factors for unsuitability to drive.

RESULTS: 738 of the 835 subjects were declared fit to drive; 342 of them were given a temporary license for 3 - 6 months and 27 received a 1-year temporary license.

CONCLUSIONS: Integrating clinical and laboratory diagnostic criteria has a strong (positive or negative) predictive value in defining fitness to drive after a drunk-driving episode, and for the purpose of regranting a driving license.

The long-term crash involvement of unlicensed drivers and riders in Queensland, Australia

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OBJECTIVE: Australian and international research has consistently found that unlicensed drivers and motorcycle riders are over-represented in serious crashes, and that these crashes are more likely to involve high-risk behaviours like drink driving and speeding. This paper reviews the long-term crash involvement of unlicensed drivers and riders in the Australian state of Queensland, with particular attention given to the key contributing factors and circumstances of these crashes.

METHOD: Crash unit level data for motor vehicles (i.e. excluding pedestrians and bicyclists) involved in all police-reported crashes for the ten year period 1995-2004 were obtained from the Queensland Government's Road Crash Database. The data included: the age, gender, licence status and type of vehicle driven for all motor vehicle controllers (i.e. drivers and motorcycle riders) involved in crashes; the circumstances of the crash; and the police-reported contributing factors.

RESULTS: Over the ten year period, the involvement of unlicensed controllers in reported crashes remained relatively stable. This group consistently represented between 3% - 4% of all controllers involved in total crashes, and between 6% - 10% of those involved in fatal crashes, confirming the over-representation of unlicensed controllers in more serious crashes. However, the proportion of unlicensed riders involved in motorcycle crashes was more variable and higher than was the case for unlicensed drivers, at all crash severity levels. For example, during the period, unlicensed riders accounted for between 7% - 14% of motorcycle riders involved in total crashes and 9% - 30% of all those involved in fatal crashes.

After accounting for certain changes in police reporting practices, the involvement of key contributing factors in the crashes involving unlicensed controllers also appears relatively stable. For example, among those unlicensed controllers involved in serious casualty crashes, 23% - 33% had alcohol or drugs in their system (compared to 3% - 7% for licensed controllers), 10% - 14% were judged to be speeding (compared to 2% - 3% for licensed controllers), and 25% - 34% were judged to be inattentive/negligent (compared to 17% - 19% for licensed controllers). Although more variable over time, unlicensed controllers were also consistently over-represented in single vehicle crashes compared to their licensed counterparts.

CONCLUSIONS: Together, the findings of this study demonstrate that both unlicensed drivers and riders remain a concern for road safety. The relative stability in their crash-involvement patterns suggests that more targeted countermeasures are required to better address this problem. In particular, unlicensed riders represent a special sub-group of concern.

LC-MS applications in forensic toxicology
Applications de la LC-MS en toxicologie médico-légale

Analytical approaches in impaired driving toxicology

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Driving under the influence of drugs - whether prescribed medication or illegal substances - is an issue of growing concern in industrialized countries as a risk and a cause for road accidents and is considered to be as dangerous as driving under the influence of alcohol. In forensic toxicology, the increasing number of samples for determination of drugs in blood is mainly due to zero tolerance laws in several countries and due to police officers, who have been trained to reliably recognize drivers under the influence of drugs (1,2).

Immunoassays tailored for a limited number of drugs (of abuse) are usually applied roadside or in the laboratory for distinction between presumptively positive and negative samples. However, many other common drugs such as anaesthetics, antiepileptics, antihistamines, opioids, psychotropics, sedative-hypnotics, can also impair drivers, but cannot be screened for by immunoassay (3). Therefore, this presentation will focus on hyphenated mass spectrometric techniques, particularly gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) with different mass analyzers such as quadrupole, ion trap, or time-of-flight analyzers (4-6). They are indispensable tools also the field of driving under the influence due to their universality, high sensitivity and specificity. They are used for confirmation of immunoassay results, for more or less comprehensive screening, library-assisted identification, and quantification of drugs and their metabolites in blood, urine, oral fluid, hair, etc. State of the art techniques as well as more or less promising upcoming technologies will be presented and their pros and cons critically discussed.

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Calibration options for triple quadrupole LC-MS data

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AIMS: Calibration with LC-MS data may be accomplished with different combinations of curve fit, weighting, and origin treatment. Agilent's Mass Hunter Quantitative Analysis software contains a calibration curve fit assistant, which allows comparison of over 100 different calibration options. Curve fit choices include linear, quadratic, power, logarithmic, second order logarithmic, and average of response factors. Weighting choices include equal, inverse x or y, inverse^2 x or y, and logarithmic. Origin choices include ignore, include, and force. The calibration curve assistant ranks calibration options by r^2, standard error, or maximum % residual error. We used the curve fit assistant to compare various calibration modes while validating an analysis of zopiclone and benzodiazepines in blood.

METHODS: Data are from the validation of the analysis of 16 benzodiazepines and zopiclone from blood extracts, acquired on an Agilent 6410 QQQ LC-MS. Blood calibrators of 25, 50, 125, 250, and 500 ng/mL, linearity check samples at 10 and 1000 ng/mL, and a control blood specimen were extracted and analyzed on multiple occasions. Endpoints included accuracy and precision of extrapolated values for the linearity check samples, and precision for the control. Although the software allowed disabling selected points, only calibrations utilizing all data points were evaluated.

RESULTS: Using data from this study, r^2 was not a sensitive measure of linearity or goodness of fit for calibration data. Maximum % residual error was a useful measure for ranking calibration curve options. As expected, inverse weighting was preferable to equal weighting for linear curve fits. When calibration data deviated from linearity, extrapolation from a linear calibration curve, below the lowest calibrator, gave inaccurate results. Several nonlinear curve fit options demonstrated low maximum residual error, and good accuracy for extrapolated values.

CONCLUSIONS: Mass Hunter's calibration curve assistant provides extensive data for evaluation of calibration options, which are useful for method validation. Inverse weighting is generally preferable to equal weighting of linear calibration curves, because of data heteroscedasticity. When linear curve fitting is applied to nonlinear chromatographic data, extrapolation below the lowest calibrator gives inaccurate results. Properly validated nonlinear curve fits may be useful in some QQQ LC-MS applications.

Detection and quantitation of anabolic steroids by LC-MS/MS

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AIMS: The use of anabolic steroids in sports is prohibited by World Anti-Doping Agency. Most of the methods for the detection of steroids are currently based on GC/MS analysis. These methods involve lengthy extraction procedures followed by derivatization of steroid metabolites. Additionally, GC/MS methods are used to detect steroid metabolites instead of parent compounds due to their lower sensitivity. We have recently developed an LC-MS/MS method for the detection, identification and quantitation of anabolic steroids in urine samples.

METHODS: Using an Applied Biosystems LC-MS/MS 3200 QTRAP system, we were able to identify several anabolic steroids of abuse (boldenone, methenolone, methandienone, nandrolone, stanozol, mesterolone, norethandrolone and androstenedione) with simultaneous quantitation of testosterone and epitestosterone levels to determine the T/E ratios. Using this method, we were able to detect and quantitate parent compounds of boldenone, nandrolone and stanozol in urine samples of actual steroid users. Briefly, the method involved hydrolysis of glucuronated steroids from urine samples with β-glucoronidase (from H. pomatia) followed by liquid/liquid extraction. The extracts were injected on to a column (C18 reverse phase, 50 mm x 4.6 mm, 3μ) using a Shimadzu autosampler and the anabolic steroids were separated by gradient elution (ammonium acetate/methanol/acetic acid). Two MRM (multiple reaction mode) transitions were monitored for each steroid using positive electrospray ionization (ESI) coupled to an MS/MS detector. D3- Stanozol was used as an Internal Standard. These studies involved the determination of LOD, LOQ and ULOL. Accuracy and precision studies were also conducted.

RESULTS: The limit of quantitation (LOQ) for most of the steroids by LC-MS/MS were found to be ~0.5 ng/mL in urine on the basis of a signal to noise ratio >10. This corresponds to 250 pg on column. The ULOL for different steroids ranged from 800-1000 ng/mL. The precision at the cutoff (10 ng/mL) was found to be within a 10% coefficient of variation and the accuracy was > 90%. We did not observe any ion suppression for these steroids as a result of the urine matrix.

CONCLUSIONS: Our studies suggest that LC-MS/MS provides a unique opportunity to detect and identify both parent steroid compounds and their metabolites in urine samples with the greatest sensitivity. Presently, we are in the process of expanding the steroid panel to include other anabolic steroids and their steroid metabolites in urine samples.

Using lLC/triple quadrupole mass spectrometry for rapid quantitation of immunosuppressants in blood

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AIMS: Immunosuppressants are used to suppress the immune system of organ transplant recipients and thus reduce the risk of organ transplant rejection. Because of their narrow therapeutic concentration range, accurate drug monitoring of these compounds is required. A fast and sensitive technique for confirming immunosuppressants in blood using the LC-MS/MS is presented.

METHODS: Blood (200 µL) and internal standard (everolimus, 100 ng/mL) is extracted with 800 µL of 0.2 M $ZnSO_4$/MeOH (30:70 v/v). After centrifugation, the supernatant is transferred to a sample vial and injected onto an Agilent 6410 LC/QQQ MS. Separation is achieved on an Agilent Series Rapid Resolution system using 0.1% acetic acid v/v in a 48:52 water/acetonitrile mobile phase (flow rate: 0.45 mL/min) on a Zorbax XDB-CN, 2.1 x 150 mm, 3.5 µm-particle size column. Ionization is carried out in both positive and negative ion polarities by electrospray with an overall run time of 3 minutes using the acquisition parameters for MRM transitions noted below:

Compound	Precursor ion	Product ion	Frag (V)	CE (V)	Dwell (msec)
Tacrolimus	826.4 (M + Na)+	616.2	200	40	150
Sirolimus	936.4 (M + Na)+	409.2	360	60	150
Everolimus (IS)	980.7 (M + Na)+	389.4	360	60	150
Cyclosporin A	1200.7 (M - H)-	1088.6	260	30	250

RESULTS: The analysis of sodium adducts for tacrolimus and sirolimus and the deprotonated form of cyclosporin A appear to provide the best sensitivity. As background levels of sodium vary, 50 µM sodium acetate is recommended for the mobile phase to ensure consistent results. Overall results are tabulated below:

Compound	Linear Range (ng/mL)	(R^2)	LOQ (ng/mL) based on a peak area %RSD	LOD (ng/mL) S/N = 3:1
Tacrolimus	0.1 – 100	0.997	0.5 - %RSD 11.3	0.1
Sirolimus	0.1 – 150	0.999	0.5 - % RSD 13.6	0.2
Cyclosporin A	1 – 5000	0.997	1.0 - %RSD 8.0	0.2*

** calculated*

CONCLUSIONS: The LC-MS/MS procedure just described is sensitive and fast, making it an excellent candidate for high throughput analysis of immunosuppressants in blood. The cyano column gives nice resolution at the flow rate of 450 µL/mn for polarity switching between positive and negative modes in analyzing the different compounds. The application of this method to clinical patient samples still needs to be investigated.

Direct screening of diuretics in human urine by LC-ESI-MS/MS with information dependent acquisition

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AIMS: Diuretics are a class of compounds largely used for either therapeutic (edema, hypertension, etc.) or illegal (doping) purposes. Probably owing to the substantial variety of their chemical structures, which makes them hardly extractable from a biological matrix in a single procedure, a relatively short list of screening methods can be retrieved in the literature. The aim of this study was to develop a LC-MS/MS method able to screen for various chemical classes of diuretics after direct injection.

METHODS: A screening procedure for 24 diuretics based on the direct injection of urine (after 50-fold dilution) by LC-ESI-MS/MS (Applied Biosytems 4000 QTrap) and Information Dependent Acquisition (IDA) allowed the acquisition of one selected reaction monitoring (SRM) transition for each compound. This triggered the acquisition of the enhanced product ion (EPI) spectrum (IDA parameters: acquisition of 1 or 2 ions whose peak height exceeded 100 counts; exclusion

for 60 seconds after 5 acquisitions of the same ion). Two subsequent injections were performed for each sample using a 10-port valve, two C18 columns (100 × 3 mm i.d., 3 µm particle size) and gradient elution (from 100% of 0.1% formic acid to 100% of acetonitrile in 12 min, 3 min at 100% acetonitrile). The first injection was eluted on column 1 and detected in negative ionization IDA; the second one was run on column 2 and positive ionization IDA.

RESULTS AND CONCLUSIONS: The sensitivity of SRM permitted to minimize sample preparation (1:50 dilution) and reach limits of detection between 0.002 and 0.25 mg/L (being 0.25 mg/L the Minimum Required Performance Limit of World Anti-Doping Agency); at the same time, ion suppression (investigated by both peak areas comparison in water vs. urine samples and by post-column infusion) was not found to significantly influence the analysis. The method was applied to urine samples from patients in treatment with diuretics at the Nephrology Department of the local Hospital. EPI spectra were stored in a library and the procedure was able to recognize by library matching various diuretics in real positive samples thus achieving a higher selectivity of detection (with full scan library search-based identification) than the usual approach based on the SRM of 2 transitions per analyte. A sample throughput of 1.5 samples per hour including processing, instrumental analysis and reporting of results was achieved.

Simultaneous detection, confirmation, and (semi-)quantification of common drugs of abuse

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AIMS: Rapid detection, identification, and quantification of drugs in biological matrices are important aspects of forensic toxicology. LC-MS/MS has proven to be a useful analytical technique because of its selectivity, sensitivity, and amount of information that can be obtained in a single run.

METHODS: Compounds analyzed were those included in the US Federal Drug Testing "SAMHSA 5" Panel: codeine, morphine, amphetamine, methamphetamine, PCP, benzoylecgonine, and THC-COOH. Urine samples were diluted 1:1 with mobile phase and injected for analysis on an LC interfaced to a hybrid triple quadrupole/linear ion trap mass spectrometer. Separation was achieved on a 2.1 mm x 50 mm Phenomenex Hydro RP column and the mobile phases were water and acetonitrile with 0.1% formic acid added to each. A quick gradient was used and total run time was 8 minutes.

Several different MS/MS scan modes were utilized to investigate the advantages and limitations of each. Methods consisted of: single period, dedicated MRM; multi-period MRM; and MRM-IDA (information dependent acquisition). MRM experiments were used for confirmation and quantitation. Two MRM transitions per analyte were monitored and a ratio of their peak areas was used for analyte confirmation. MRM-IDA experiments consisted of an MRM survey scan to detect the presence of a target analyte. For the MRM-IDA survey scan, one MRM transition was used for each analyte. If a signal was detected in a transition, a linear ion trap full scan MS/MS spectrum was acquired.

A list of MRM transitions for each analyte is shown below:

	Quantifier		*Qualifier*	
Analyte	Q1	Q3	Q1	Q3
Amphétamine	136	91	*136*	*119*
Methamphetamine	150	91	*150*	*119*
Codeine	300	115	*300*	*152*
Morphine	286	152	*286*	*165*
6-MAM	328	152	*328*	*211*
Benzoylecgonine	290	168	*290*	*105*
Phencyclidine	244	91	*244*	*86*
COOH-THC	345	299	*345*	*193*

RESULTS AND CONCLUSIONS: For quantitative analysis using dedicated MRM experiments, LLOQs for all analytes were 5 ng/mL or better with a linear dynamic range of at least 2.5 orders of magnitude. Two MRM transitions, a quantifier and qualifier, were used for each analyte and the ratio calculated for confirmation. Less than 5% of the calibrators and QC samples had a ratio that was not within 20% of the acceptable value. Precision and accuracy for calculated concentrations were better than 15% when a single period experiment was used and better than 10% when the experiment was divided into chromatographic periods.

When MRM-IDA was utilized, qualitative screening and confirmation were acquired in a single analysis. For confirmation via MS/MS spectral library search, it was necessary to have concentrations of at least 10 ng/mL to have a sufficient number of ions to obtain a quality MS/MS spectrum and the LLOQ was slightly higher (~3x) than versus dedicated MRM method. Full scan MS/MS provides a higher degree of certainty versus an MRM ratio. Semi-quantitative information could be obtained using the MRM traces, which yielded precision and accuracy around 30%. This estimate of analyte concentration obtained from the screening experiment

could be used to adjust sample preparation – size, dilution, etc. – before running a dedicated quantitative experiment to obtain true quantitative results.

New ESI-TOF technology enabling screening of an unlimited number of known and unknown compounds in different matrices

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AIMS: Classical target pre-selected LC-MS analysis in MRM mode allows the screening of only a limited number of targets simultaneously. All possible targets must be taken into account when setting up the experiment, and retrospective screening for fresh targets after the run is not possible. Since the number of targets is increasing continually, alternative approaches for non-presumptive screening are desirable without making compromises in sensitivity.

METHODS: A new design of ESI-TOF mass spectrometer is presented which fulfils all these criteria. As full scan spectra are acquired, the information of an unlimited number of targets is always available. This new generation of ESI-TOF instruments combines this advantage with a high sensitivity in the sub-ppb to low ppb range. The mass accuracy (3-5 ppm) and resolution (15,000) of such a device permits highly discrete separation of a target from matrix, to a tolerance as exacting as 2 millidalton chromatograms.

The latest developments of this type of mass spectrometer introduced a second analytical dimension. In addition to the high mass accuracy, the use of the precise isotopic pattern gives a high confidence in the characterization of a compound (the isotopic pattern is characteristic for every molecular formula). In combination with the high mass accuracy even unknown compounds can be identified with a high certainty.

An integral part of the presentation is the technical principle of this new ESI-TOF technology. We acquired LC-MS data from urine samples, oral fluids, and salt-rich buffers spiked with up to 22 drugs of abuse and pharmaceuticals in concentrations ranging from 1.0 ppb to 10 ppm (e.g. codeine, morphine, amphetamines, cocaine and others). In addition results of real case samples from hair and blood are part of the talk. All separations were done with an Agilent 1200 HPLC system, mass spectra were acquired with a micrOTOF from Bruker Daltonik. As column we used Phenomenex, Synergi 2.5 µm, Fusion RP100, 100 x 2 mm, with a Phenomenex, Gemini C18, 4 mm x 2 mm precolumn. Prior to LC-MS analysis, the samples were cleaned up by solid phase extraction *(Rapid Communications in Mass Spectrometry, 2006, **20**, 1161-1167)*. These results were compared with data from "dilute-and-shoot" experiments. It turned out that the cleanup procedure increases the sensitivity up to a factor of 10.

RESULTS: The information content of a "precise-mass-plus-isotopic-pattern" versus "precise-mass-only" approach is compared. When all possible molecular formulas of flurazepam are calculated within 5 ppm mass accuracy by allowing more than 10 carbon atoms and up to 1 bromine, chlorine and fluorine to be present, the result list contains 15 hits. Just based on the mass accuracy, all of them have the same probability to be correct. When the isotopic pattern is added as 2nd criterion, the correct molecular formula is clearly the top hit with the best isotopic match (here: mass accuracy with external calibration 2.1 ppm).

Isotopic pattern plus mass accuracy are also helpful when retention time shifts due to matrix effects. In the case of methadone we observe 4 peaks with the m/z 310.217 +/- 0.02 Th. These 4 peaks elute all within 3 minutes, all differ from the reference retention time by up to 2.5 minutes. The correct molecular formula of $C_{21}H_{27}NO$ could only be found in one LC-peak. The other 3 compounds have different elemental compositions.

Drug effects on drivers

Effets des médicaments psychotropes sur les conducteurs

Driving under the influence of gamma-hydroxybutyrate (GHB)

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This presentation compares the ages, gender and concentrations of the illicit depressant drug gamma-hydroxybutyrate (GHB) in blood samples from people apprehended in Sweden for driving under the influence of drugs (DUID). Results for DUID suspects were compared with people arrested for petty drug offences involving the use of illicit drugs (non-traffic cases).

We used a forensic toxicology database (TOXBASE) to search for all occurrences of GHB in forensic blood samples between the years 1998 and 2006. GHB was determined in blood samples by gas chromatography (GC) with a flame ionization detector and gamma-valerolactone was used as the internal standard. Blood-

proteins were precipitation by adding acetone and the supernatant, after centrifugation, was acidified to convert GHB into gamma-butyrolactone (GBL). The GBL produced was extracted into dichlormethane and an aliquot injected directly into the gas chromatograph, which was fitted with a capillary column and DB-5 as the stationary phase. This method gave a linear response to GHB concentrations in blood ranging from 8 to 1000 mg/L and the cut-off concentration for reporting a positive result was 8 mg/L.

The mean and median concentrations of GHB in 473 cases of DUID were 90 and 84 mg/L, respectively and the highest was 340 mg/L. There was only a weak association between the concentration of GHB in blood and the driver's age (r = 0.16). The DUID suspects were mainly men (96%) with an average age of 26 y (range 15 - 50 y). GHB was the only drug present in 185 cases (39%) and the mean and median concentrations were 92 mg/L and 85 mg/L, respectively. People apprehended for using illicit drugs (non-traffic cases) (N = 1061) had slightly higher concentrations of GHB in blood (mean 118 mg/L, median 110 mg/L). In non-traffic cases there was also a higher proportion of women (12%), although the mean age of offenders was about the same as for the DUID suspects (26 y). The signs of drug influence noted by the arresting police officers included agitation, unsteady gait, slurred speech, irrational behaviour, jerky body movements, dilated pupils and spitting.

GHB is cheap, easy to obtain and to administer and is a drug mainly used by young people (average age 25 - 26 y). GHB is a powerful depressant of the central nervous system and causes marked impairment of skills important for safe driving. Interpreting the concentrations of GHB in blood is complicated owing to the short half-life (30 - 40 min), which means that the concentrations reported here are appreciably less than those existing 1 - 2 hours earlier, such as at the time of driving.

Methadone and impairment in apprehended drivers

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OBJECTIVE: Methadone is a potent opioid receptor agonist used in the treatment of opioid dependence. Approximately 4,000 patients enrolled in rehabilitation programs for heroin addiction, in Norway, received methadone by the end of 2006. In addition, illegal misuse of methadone, although of unknown magnitude, is a well-known problem. According to Norwegian guidelines, patients who are in rehabilitation programs are permitted to drive a motor vehicle provided, firstly, that they have been stabilised on a fixed dose of methadone for a period of at least 6 months, secondly, that the treating doctor finds them fit, and thirdly, that no other drugs are used. The purpose of this study was to investigate apprehended drivers who had; 1) methadone as the only drug in their blood at the time of apprehension, 2) methadone in the presence of other psychoactive drugs in their blood at the time of apprehension. Lastly, it was also desirable to study the relationship between blood methadone concentration and impairment as measured by clinical test for impairment (CTI).

METHODS: The division of Forensic Toxicology and Drug Abuse (DFTDA) at the Norwegian Institute of Public Heath analyses blood samples from all drivers suspected of driving under the influence of drugs. Cases with positive results for methadone in blood were collected over the period 2000 to 2006.

RESULTS: 666 cases of drugged driving with methadone were identified from a total of approximately 55,000 for the period 2000 to 2006. The majority of drivers were men (> 80%), aged between 30 and 40 years. No significant difference in methadone concentration was found for sex. Methadone was the only psychoactive drug detected in blood in only 11 cases. A statistically significant ($p < 0.01$) difference in blood methadone concentration was found between cases where only methadone was detected (median 0.46 mg/L (range 0.19–0.65)), and cases where methadone was detected in combination with other psychoactive drugs (median 0.28 mg/L (range 0.06 – 0.24)). CTI was carried out in 613 of the cases. Interestingly, a concentration-impairment relationship was not seen for these cases. Approximately 90% of cases had at least one benzodiazepine present in blood (most commonly flunitrazepam), while the most frequently observed other substances were amphetamine (in 189 cases), Δ^9-tetrahydrocannabinol (THC, in 191 cases) and morphine (in 163 cases), in descending order.

CONCLUSIONS: Cases of driving impairment involving methadone alone were very rare (only 11 over a 6 year period), while methadone in combination with other drugs was more common; in descending order of frequency, benzodiazepines (flunitrazepam, diazepam, clonazepam etc.), amphetamine, THC and morphine. No relationship between methadone concentration and impairment as judged by CTI was seen for either methadone-only cases, although these were very few, or cases involving methadone and other psychoactive drugs.

The distribution of oxazepam and oxazepam glucuronide in body fluids after a single dose of oxazepam and the influence on four standardized field sobriety tests

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BACKGROUND AND OBJECTIVES: In the Netherlands, oxazepam is the most frequently prescribed benzodiazepine. Driving under the influence of oxazepam may be a public health problem. Determination of the time windows of detection of oxazepam and its metabolite in blood, serum and oral fluid is needed in order to study the feasibility to establish legal limits. The aims of this study are to determine the concentration-time profile of oxazepam and its metabolite in oral fluid, to relate this to the blood and serum profiles and to explore the dose – performance relationship of oxazepam in four standardized field sobriety tests.

STUDY DESIGN: Eight healthy male volunteers completed a double blind crossover study. The subjects received in random order 15 mg and 30 mg oxazepam orally, on 2 days separated by 1 week in order to exclude carry-over effects.

METHODS: Blood (B), serum (S) and oral fluid (OF) samples were collected up to 8.5 h (blood, serum) or up to 48 h (oral fluid) after administration and assayed for concentrations of oxazepam and oxazepam glucuronide. The concentration-time profiles in serum, whole blood and oral fluid were fitted by using MwPharm® 3.50. Four standardized field sobriety tests were performed in order to study the relation between dose and performance. Before intake of oxazepam, a blood and oral fluid sample were taken and the tests were performed to establish pre-drug values.

RESULTS: Concentrations of oxazepam in blood and serum were comparable (mean ratio B/S = 0.90; range 0.83 - 0.97). Concentrations of oxazepam in oral fluid were low: the mean OF/B ratio was 0.05 (range 0.04 - 0.07) and the mean OF/S ratio was 0.04 (range 0.03 - 0.07). Concentrations of oxazepam glucuronide were higher in serum than in blood (mean ratio B/S = 0.64; range 0.46 - 0.81). Concentrations of oxazepam glucuronide in oral fluid were very low: the mean OF/B ratio was 0.004 (range 0.002 - 0.006) and the mean OF/S ratio was 0.002 (range 0.001 - 0.004). Influence of the single doses of oxazepam on the Walk and Turn Test, One Leg Stand Test, Finger to Nose Test and the Romberg Test tests was not entirely elucidated, due to possible interfering factors (e.g. practice, fatigue early in the morning) and the unknown sensitivity of the tests for oxazepam.

CONCLUSIONS: In oral fluid, both oxazepam and oxazepam glucuronide were detected. Oral fluid was tested positive for oxazepam at least 8.5 hours after intake of a single dose of 15 or 30 mg oxazepam. The window of detection of oxazepam glucuronide depended very much on the analytical detection limit and could not be established in all cases. The concentration-time profiles of oxazepam in oral fluid ran parallel to those in blood and serum. The presence of oxazepam in oral fluid is probably a good indicator of recent use and may be indicative of driving under the influence.
More research (e.g. laboratory tests, driving tests) has to be done to explore the concentration – impairment relation.

Residual effects of hypnotics on actual driving of healthy young volunteers

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OBJECTIVE: Residual sedation the morning after bedtime use of hypnotics is a major problem with respect to traffic safety. Gaboxadol is a selective extrasynaptic $GABA_A$ agonist (SEGA) intended for the treatment of insomnia. It acts primarily by activating benzodiazepine-insensitive extrasynaptic $\alpha_4\beta_3\delta$-, and $\alpha_6\beta_3\delta$-containing $GABA_A$ receptors involved in tonic inhibition. After oral doses the drug is rapidly absorbed (tmax approximately 30 min) and eliminated ($t_{1/2}$ 1.5 - 2.0 hours). Clinical trials did not reveal any residual effects on cognitive functioning of bedtime doses up to 20 mg. The hypothesis of the study was that gaboxadol 15 mg is free of residual effects on driving the morning after bedtime administration, and that it may be safe for use later in the night.

METHODS: Twenty-five healthy volunteers (13 men and 12 women, mean ±SD age 31.4 ± 1.5 years) participated in a double-blind, placebo controlled, 5-way crossover study. They ingested capsules twice on each treatment night; once at 23:00 hours before initiating sleep and again after being briefly awakened 5 hours later. Treatments were: one session with placebo at both times, two sessions with active treatment (gaboxadol 15 mg and zopiclone 7.5 mg) in the evening followed by placebo in the middle of the night, two sessions with placebo in the evening and active treatment in the middle-of-the-night (gaboxadol 15 mg and zolpidem 10 mg). Subjects arose at 07:00 hours. Residual drug

effects on laboratory tests (tracking, divided attention, digit symbol substitution, word learning, postural stability, subjective alertness) were assessed between 7:30 and 8:15 hours and on actual driving ability between 9:00 and 10:00 hours. The primary dependent variable was Standard Deviation of Lateral Position (SDLP in cm, an index of weaving) in the standardized highway driving test.

RESULTS: Gaboxadol 15 mg was without significant effects on driving as measured by SDLP between 10-11 hours after evening administration. Nonetheless, it had minor effects on speed variability and performance in the divided attention test. In contrast, zopiclone 7.5 mg significantly impaired driving. The effects on SDLP were comparable to those found previously for alcohol while BAC was 0.05 g/dL. In addition zopiclone significantly impaired performance in all laboratory tests, except tracking. Remarkably, subjects did not feel significantly less alert in the morning, as compared to placebo. The middle of the night doses of zolpidem 10 mg and gaboxadol 15 mg both impaired driving between 5 to 6 hours after administration. The effects of zolpidem 10 mg were most pronounced. In addition it impaired all laboratory performance parameters. Gaboxadol, on the other hand, had significant effects on psychomotor performance, but not on memory. Subjects felt significantly less alert and contented following both middle of the night doses of hypnotics.

CONCLUSIONS: Gaboxadol 15 mg is unlikely to produce residual effects on driving between 10 and 11 hrs after evening administration. However, patients should be warned not to drive within 6 hours after use of gaboxadol 15 mg later in the night, as is the case for zolpidem 10 mg. Interestingly, gaboxadol seems to be the first GABAergic hypnotic that does not impair memory. Finally, patients should be warned not to drive within 11 hours after bedtime use of zopiclone 7.5 mg, in particular because they seem unaware of residual sedation of this drug themselves.

Effects of alprazolam 1 mg on cognition and driving performance: a comparison between immediate and extended release formulations

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OBJECTIVE: Alprazolam is the most frequently used benzodiazepine in the treatment of panic disorder and anxiety. It is available in an immediate release (IR) formulation and an extended release (XR) formulation. Alprazolam XR produces peak plasma concentrations that are about 50% of a similar dose in IR formulation, and occur between 5 and 12 hours following administration. Peak plasma concentrations following IR formulations are reached within 0.7 to 1.8 hours. The present study aimed to compare the effects of single oral doses of 1 mg alprazolam IR, 1 mg alprazolam XR and placebo on driving ability and cognition.

METHODS: Eighteen healthy volunteers (9 males, 9 females) with a mean (± SE) age of 32.3 (± 2.0) years (range 20 - 45 years) participated in a double blind, placebo-controlled, three-way crossover study. Between 4 and 5 hours post dose, subjects performed a standardized driving test on a primary highway in normal traffic. The primary dependent variable in this test is Standard Deviation of Lateral Position (SDLP in cm, an index of weaving). Effects on attentional resources and inhibitory control were assessed at 1, 2.5 and 5.5 hours post dose, using a divided attention test and stop signal test. Effects on memory were measured only 1 hour after administration, using a word learning test. In addition, serum concentrations were determined from blood samples collected at 1 and 6 hours after ingestion of the drugs.

RESULTS: Both alprazolam 1 mg formulations significantly impaired performance in the standardized highway driving test. Ten driving tests were terminated prematurely because the driving instructor judged the subject to be too drowsy to continue safely: seven rides after IR formulations and 3 rides after XR formulations. Mean SDLP was increased by 8.2 cm after IR and 3.9 cm after XR formulations, as compared to placebo. Laboratory test results were in line with the driving data. Mean (SE) serum concentrations at 1 and 6 hours after administration of alprazolam IR were 4.9 (1.0) µg/L and 10.6 (0.5) µg/L, and after alprazolam XR 1.7 (0.2) µg/L and 9.0 (0.6) µg/L, respectively.

CONCLUSIONS: The impairing effects of the alprazolam 1 mg XR on driving and cognition were generally less after administration of the extended release formulation as compared to its immediate release equivalent, but still of sufficient magnitude to sharply increase the risk of becoming involved in traffic accidents.

An examination of 1,1-difluoroethane in traffic cases

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1,1-difluoroethane (DFE), also known as Freon 152A, is a halogenated hydrocarbon used as an aerosol propellant in household products used to remove dust from electronic devices. Occasionally DFE is used

as a refrigerant and can also be found in adhesive removers and correction fluids. DFE is also abused by individuals who intentionally inhale the compound to achieve feelings of euphoria. Effects are similar to other common inhalants and include ataxia, disorientation, dizziness, nausea and visual hallucination.

At present there is very little literature on the topic of DFE use and driving. There are currently two separate reports of fatal accidents involving the drug. The first was reported by Broussard et al (1997). An eighteen year old male driver and his seventeen year old male passenger crossed the median of a four-lane highway and collided with a minivan. The driver of the minivan suffered serious permanent injuries. The causing driver and his passenger both died at the scene. A DFE concentration of 78 mg/L was detected in the eighteen year old male driver. An ethanol concentration of 0.013 g/100mL was also detected in the blood of the driver. His passenger had a DFE concentration of 35 mg/L. A can of airbrush propellant was located in their car. The second report of DFE having a role in driving impairment was reported by Hahn et al (2006). A twenty-four year old female was reported to have been huffing a can of compressed air while driving her motor vehicle. She became irate and began to drive erratically at a high rate of speed until losing control of the vehicle and colliding with a telephone pole. The driver died at the scene and her passenger was treated for minor injuries. The driver had femoral blood concentrations of 29.8 mg/L (other tissues were also quantitated).

We report eight cases of difluoroethane detection in drivers tested by the Washington State Toxicology Laboratory from 2003-present. DFE positive cases are detected by Headspace GC and confirmed by GC-MS. Five of the subjects are male and one subject is female (two men were involved in more than one case). The ages of the subjects range from seventeen to thirty-two years old. Six of the cases were submitted as the result of motor vehicle accidents, one was submitted after the subject was found passed out behind the wheel of his vehicle, and one case was submitted after the driver was arrested for erratic driving. Of those involved in motor vehicle accidents a common trend is that the drivers claim no recollection of the events leading to the accident.

References:

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Advances in analytical toxicology methods

Développements récents en toxicologie analytique

Development of an isotope-dilution LC-MS/MS method for quantitative detection of THC-glucuronide in urine of Cannabis consumers

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AIMS: In several studies it has been shown that THC-glucuronide (THC-glu) is a phase II metabolite of delta-9-Tetrahydrocannabinol (THC) found in human urine. GC-MS has been used for its indirect determination, which either requires derivatization prior to measurement or quantification of THC obtained by complete hydrolysis of THC-glu. To avoid such sample pretreatment, LC-MS/MS is the method of choice. Based on IDMS (Isotope Dilution Mass Spectrometry) as the measurement principle, an LC-MS/MS method has been developed that has the potential of being used as a primary method of determining THC-glu, in, for example, value assignment in reference materials.

METHODS: Isotopically labeled THC-glu required as an internal standard was obtained by enzymatic glucuronidation of deuterated THC (THC-D3) using an UDP-glucuronosyltransferase (UGT). The UGT-glucuronidation assay was performed at 37°C in Tris-Buffer Solution (50 mM, pH = 7.5) containing Reaction Mix Solutions A (UDPGA Cofactor) and B (5xUGT Buffer Mix with Alamethicin) and stopped after 24 hours. After separation from protein, the THC-glucuronide-D3 product was further purified using semipreparative HPLC.

A solution of commercially available THC-glu, (isotopically natural form) has been value assigned by hydrolysis- GC/MS as a reference solution for calibration of the LC/IDMS assay. After addition of internal standard to 1mL urine and 1mL 0.25M acetic acid, THC-glu was extracted by liquid-liquid extraction with 2 mL tert-butyl methlyether. The solvent was evaporated under N_2 and the residue was dissolved in HPLC solvent (ACN/H_2O) and injected into the LC/MS-MS system.

Multiple reaction monitoring (MRM) of the transition m/z 489.2 $\rightarrow$ 313.2 was used in the negative mode of an ESI-LC-MS/MS (triple quad mass spectrometer 4000 Q Trap, Applied Biosystems) for the detection of THC-glu.

RESULTS: The method has a limit of detection (LOD) of 0.15 ng/mL and a limit of quantification (LOQ) of 1 ng/mL in urine with good linearity in the range 0 to 10 ng/mL for the glucuronide (R=0.999). Intra- and interday precision demonstrated CV's of 3% for both. Practical applicability and performance have been evaluated by determination of THC-glu in selected urine samples of cannabis consumers.

CONCLUSIONS: The enzymatic reaction procedure is a way to obtain isotopically labeled THC-glu in solution that can be used for quantitative analysis by isotope dilution mass spectrometry (IDMS). The LC/MS-MS method has proven to be selective and sensitive to detect THC-glu in human urine.

An automated solid-phase extraction LC-MS/MS system for the analysis of cocaine and metabolites in blood and urine

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AIMS: To present a novel analytical procedure for cocaine and its major metabolites utilizing a fully automated solid-phase extraction (SPE) system coupled to a liquid chromatograph-tandem mass spectrometer (LC-MS/MS).

METHODS: This is a unique automated method in that it interfaces the Spark Holland SymbiosisTM to the Applied BioSystems 4000 QTrap® so that these two instruments function as a single, unified system. The Symbiosis™ consists of a storage compartment, autosampler, two LC pumps and an extraction unit. This system allows for the direct extraction of urine and blood samples with elution into the stream of the mobile phase for chromatographic separation before mass spectral detection. The Applied BioSystems QTrap® is a hybrid triple quadrupole/linear ion trap mass spectrometer.

The method was developed and optimized for the extraction of cocaine (COC), benzoylecgonine (BE), cocaethylene (ECOC), ecgonine methyl ester (EME), and ecgonine (E) from whole blood and urine. Deuterated analogues of these analytes (d_3, except d_5BE) were used as internal standards. Following simple sample preparation steps of centrifugation and filtration, the samples were placed directly on the SPE-LC-MS/MS system for automated analysis.

Ten solid phase cartridges were evaluated to determine the optimal SPE material for the extraction of all analytes from blood and urine. The evaluated cartridges were CN, C2, C8, C8 EC (Encapped), C18, C18 HD, Hysphere resin SH (Strong hydrophobic), Hysphere resin GP, Hysphere MM Cation (Cation exchange), and Hysphere MM Anion (Anion exchange). The solvents used for the SPE procedure were thoroughly investigated to determine the optimal combination to eliminate matrix components that may cause analyte ion suppression.

Several C8 and C18 analytical columns were evaluated for best chromatographic retention and peak shape. Multiple reaction monitoring (MRM) of the product ion arising from the corresponding precursor ions was used in order to enhance the selectivity and sensitivity of the method. Sixteen channels with the precursor's m/z → product m/z values were selected and monitored.

Full method validation was conducted following the *FBI Laboratory Chemistry Unit Validation Protocol* using both whole blood and urine matrices.

RESULTS: The results demonstrated that for SPE, the C8 EC, C18, and C18 EC cartridges retained only COC, ECOC, BE and EME. The C2 and C8 cartridges extracted COC and ECOC very well, with poor retention of BE and EME. Likewise, the Hysphere MM Cation cartridge retained COC and ECOC with poor retention of BE, EME and E. The Hysphere MM Anion cartridge extracted all five analytes with excellent recovery. For chromatographic separation, the Xterra C18 was found to be optimal for all compounds studied. Likewise, several mobile phase combinations were evaluated. The results suggested that the optimal mobile phase was a gradient from 100% of 0.1% formic acid in water to 90% of 0.1% formic acid in acetonitrile. The total run time was 10 minutes, including SPE and chromatographic separation.

Validation results demonstrated absence of interferences from other analytes or matrix components due to the MRM technique employed. Further, a quadratic calibration model was determined to be most suitable for quantitative analyses. Bias, repeatability, and intermediate precision were determined by analyzing replicates of QC samples at three concentrations within the calibration range over five separate days. For all analytes, bias results were within 7% of the target concentration.

A validated LC-ESI-MS assay for the determination of mdma and its metabolites mda, hhma, and hmma in squirrel monkey plasma

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AIMS: (±)3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug that has neurotoxic potential toward brain serotonergic and/or dopaminergic nerve terminals, depending on species and treatment conditions. Pharmacokinetic data of MDMA and its metabolites may shed light on the mechanism by which MDMA produces neurotoxic effects. As studies of neurotoxic mechanisms are

best approached in experimental animals, the presented study was designed to develop a LC-ESI-MS assay for quantification of MDMA and its main metabolites 3,4-methylenedioxyamphetamine (MDA), 3,4-dihydroxymethamphetamine (HHMA), and 4-hydroxy-3-methoxymethamphetamine (HMMA) in plasma of the squirrel monkey, a non-human primate species often used in studies of MDMA neurotoxicity.

METHODS: After adding the internal standards MDMA-d_5, MDA-d_5, and pholedrine to squirrel monkey plasma samples (100 µL) preserved with $Na_2S_2O_5$ and EDTA, the samples were subjected to enzymatic conjugate cleavage (glucuronidase, 50°C, 90 min). Thereafter, 4-methylcatechol was added and plasma proteins were precipitated with perchloric acid. The precipitated proteins were separated by centrifugation (16,000 g, 5 min), and 5 µL of the supernatant were injected into the LC-MS system (Agilent 1100 series). The analytes were separated using a Zorbax 300-SCX column and an isocratic mobile phase consisting of 5 mM aqueous ammonium formate adjusted to pH 3 with formic acid and acetonitrile (70:30 v/v) with the following flow rate: 0-4 min, 0.8 mL/min; 4-11 min, 1.0 mL/min; 11-14 min, 0.8 mL/min. Detection was achieved by mass spectrometry using positive electrospray ionization, a fragmentor voltage of 100 V, and selected ion monitoring. The LC-MS assay was fully validated according to international guidelines.

RESULTS: Baseline separation of all analytes was achieved within a 14 min run time and they were all detected with excellent sensitivity. The method was linear from 20 to 1000 ng/mL for MDMA, HMMA and HHMA and from 10 to 500 ng/mL for MDA. Accuracy (bias) data ranged from -2.0% to 18.9% and those for precision (CV) from 2.2% to 12.4%. Studies on matrix effects gave no indication of major ion suppression or enhancement. All analytes were stable during three freeze/thaw cycles and over 14 h in processed samples. The assay was successfully applied to authentic squirrel monkey plasma samples allowing determination of metabolite formation and calculation of plasma concentrations.

CONCLUSIONS: The LC-ESI-MS assay presented here is the first allowing simultaneous and reliable quantification of MDMA and its metabolites HHMA, HMMA, and MDA in squirrel monkey plasma. It should be useful in acquiring reliable pharmacokinetic and toxicokinetic data in squirrel monkeys. These data may help elucidate the mechanism by which MDMA produces neurotoxic effects. [Supported by PHS Grants DA05707 and DA017964).

High throughput analysis of amphetamines in urine with on-line-solid phase extraction-liquid-chromatography-tandem mass spectrometry

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AIMS: A completely integrated on-line SPE-LC-MS/MS automated method was developed and fully validated for the direct analysis of 7 amphetamines in urine. The combination of high throughput on-line SPE with the well-known sensitivity and specificity given by the MS/MS resulted in the elimination of the bottleneck associated with the sample preparation requirements and with the higher sensitivity, accuracy and precision.

METHODS: The method involves a fully automated SPE system (Spark Symbiosis Pharma), which allows the simultaneous extraction of the second sample in one clamp and the elution of the first sample in a second clamp, as such, achieving an optimal use of the extraction time without manual transfers and pre-concentration steps. This system offers the entire process of conditioning (methanol, water and ammonium formate buffer 10 mM pH 4), sample application (to cation exchange mode cartridges), washing (ammonium formate buffer and methanol: water (50:50 (v,v))) and elution (5% ammonia in methanol), taking place at constant flow rates, yielding better precision in comparison with off-line driven extraction procedures.
Chromatographic separation was achieved using an Atlantis dC18 column, eluted with a mixture of ammonium bicarbonate buffer 10 mM pH 8 and methanol. The applied LC gradient ensured the elution of all the drugs within 16 min and produced chromatographic peaks of acceptable symmetry. Selectivity of the method was achieved by a combination of retention time, and two precursor-product ion transitions for the non-deuterated analogues.

RESULTS: The method was fully validated using only 50 µL of urine, including linearity (25 - 1000 ng/mL), intra-assay and inter-assay precision (RSD < 15%, except for MDMA which was < 19%), LOQ (25 ng/mL), LOD (ranged from 0.5 ng/mL to 2.5 ng/mL), accuracy (> 93%), matrix effects and stability studies. External quality controls (at two concentration levels) containing most of the compounds used during method validation also demonstrated the accuracy of the method.

The method was subsequently applied to authentic urine samples, previously screened with an immunoassay

technique, obtained from roadside controls, suicide attempts and from forensic and toxicology cases. The measured concentrations varied considerably.

CONCLUSIONS: The validation and actual sample analysis results show that this method is rugged, precise, accurate, and well suitable for routine analysis where more than 100 samples may be non stop analyzed in 48 hours, with a minimum sample handling.

A method for the quantification of heroin and its metabolites in plasma by liquid chromatography-tandem mass spectrometry

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AIMS: In recent years, intra-nasal heroin has been recommended as an alternative to intravenous administration for the treatment of acute severe pain in children. This provides a rapid and less painful route of administration without decreasing the effectiveness of the analgesic properties. The need for a sensitive technique for the detection and quantitation of heroin and its metabolites is essential due to low concentrations of heroin and metabolites in children's plasma, as a result of the low dose of heroin given, and the limited sample volume obtained from children (0.25 mL or less). In addition, heroin can be easily hydrolysed to 6-monoacetylmorphine (6-MAM) during sample preparation and extraction, so this must be considered when developing a solid-phase extraction (SPE) method to prevent the hydrolysis of heroin.

METHODS: 250 µL of plasma was added to 300 µL of 0.01 M ammonium carbonate, pH 9.3 and 25 µL of the internal standard working solution (1 µg/mL) was added. The mixture was vortex mixed. The supernatant was applied to a Bond Elut C18 SPE cartridge preconditioned with 2 mL methanol, 1 mL of deionised water, and 2 mL of 0.01 M ammonium carbonate (pH 9.3). The SPE cartridge was washed twice with 1 mL 0.01 M ammonium carbonate (pH 9.3), and then dried for 10 minutes. Retained drugs were eluted with 2 mL methanol, after which the eluate was evaporated to dryness under nitrogen at 50°C. The extract was recons–tituted with 80 µL of initial mobile phase and 20 µL were injected using a Thermo Finnigan LCQ DECA XP Plus ion trap instrument (Thermo Finnigan, San Jose, USA) equipped with a surveyor LC system interface. Chromatographic separation was achieved using a Synergy Polar RP column (150 x 2.0 mm, 4-µm particle size), protected by a guard column with identical packing material (4 x 2.0 mm, Phenomenex, Torrance, CA). Gradient elution was based on a mobile phase consisting of 10 mM ammonium formate adjusted to pH 3 (A) and acetonitrile (B) at a flow rate of 0.3 mL/min in the first 8 min, decreasing to 0.2 mL/min at 13 min for the next 13 min. After that, the initial flow rate was applied until the end of analysis. The gradient conditions were initially 97% of solution A for 3 min; decreasing to 84.5% at 8 min, to 74% at 13 min and to 20% at 26 min, 5% of solution A was maintained for the next 3 min before returning to 97% for 7 min prior to the next injection.

RESULTS: Intra-day and inter-day precision for all analytes were determined at three concentration 1, 5, and 25 ng/mL and these were found to be 2.5 - 13.4% and 1.8 - 15% respectively. Recoveries of analytes of interest were between 81% and 109%. Calibration curves were linear for all analytes over the concentration range 0.1 - 50 ng/mL and correlation coefficients (R_2) were better than 0.999. Limits of detection and limits of quantitation were 0.08 - 0.37 ng/mL and 0.28 - 1.22 ng/mL respectively.

CONCLUSIONS: A sensitive and specific method for the quantitation of heroin metabolites, namely heroin, 6-MAM, morphine, morphine-3-glucuronide, morphine-6-glucuronide and normorphine in human plasma was developed and validated. This method was developed for testing plasma samples obtained from children who are under treatment for acute severe pain and data of samples tested will be presented in the future.

The comparison of a new chemiluminescence immunoassay screen with a single-step enzyle linked immunosorbent assay (Elisa)

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AIMS: The current procedures used by the Toxicology Laboratory, Forensic Science South Australia, for the screening of whole blood (postmortem and antemortem) for drugs of abuse (opiates, benzodiazepines, cannabinoids and amphetamines) is a single-step ELISA screen followed by either GC-MS or LC-MS/MS for confirmation of the parent drug. Randox has recently released a new chemiluminescence immunoassay system. The principle used in this immunoassay technique is similar to other heterogeneous assays but offers greater sensitivity using chemiluminescence. The aim of this report is to compare this technique to the existing ELISA technique.

METHODS: The Toxicology Laboratory has conducted an evaluation of this new technique by comparison to results obtained from 185 whole blood samples (145 post-mortem and 40 ante-mortem) using our routine screening system (ELISA) with MS confirmation. The cut-off concentrations for the Randox Evidence drugs of abuse (DoA) assay were set at the following: opiates (10 ng/mL), benzodiazepines (10 ng/mL), carboxy-THC (5 ng/mL) and methamphetamine (20 ng/mL). All samples were subjected to our standard confirmation

methods to verify the immunoassay results. Opiates (morphine, codeine and 6-monoacetylmorphine) were analysed by solid phase extraction (SPE) followed by LC-MS/MS. Benzodiazepines were confirmed by liquid-liquid extraction followed by capillary GC-MS (thermally unstable benzodiazepines e.g. temazepam and oxazepam were confirmed by LC-MS/MS). Amphetamines (methamphetamine, amphetamine, MDMA, MDA), THC and carboxy-THC were confirmed by SPE followed by derivatisation and capillary GC-MS.

RESULTS: The following table summarises the results obtained:

	Amphetamines	Opiates	Benzodiazepines	Cannabinoids
True Positives (TP)	14.5%	20%	22.7%	14.5%
False Positives (FP)	40%(1)	6.4%	2.7%	4.8%(3)
False Negatives (FN)	10.8%(2)	1%	1.1%	ND4
True Negatives (TN)	35.1%	71.3%	68.6%	77.3%
Sensitivity	0.57	0.94	0.95	ND(4)
Specificity	0.49	0.91	0.96	0.94
Positive Predictive Value (PPV)	0.28	0.75	0.89	0.75
Negative Predictive Value (NPV)	0.76	0.98	0.98	ND(4)

(1) Six (6) samples were putrefactive effusions from decomposed bodies
(2) Eight (8) samples contained MDMA
(3) Antemortem samples only analysed for THC, may be inflated, as Carboxy-THC may be present
(4) ND - Not determined as laboratory analysis is not complete at this time

Identification of the cytochrome P450 isoenzymes involved in the formation of the main metabolites of the designer drugs DOI, DOB, MDOB, and TMA-2 and studies on their capability to inhibit CYP2D6

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AIMS: 4-Iodo-2,5-dimethoxyamphetamine (DOI), 4-bromo-2,5-dimethoxyamphetamine (DOB), 4-bromo-2,5-dimethoxymethamphetamine (MDOB), and 2,4,5-trimethoxyamphetamine (TMA-2) are designer drugs which have appeared on the illicit drug market. Meanwhile, DOB and TMA-2 have been scheduled in the German Controlled Substances Act. Because of the various possibilities of interactions between different drugs especially with respect to metabolism the first aim of our study was to identify the cytochrome P450 (CYP) isoenzymes involved in the main metabolic steps of DOI, DOB, MDOB, and TMA-2. The second aim was to check whether these drugs are capable to inhibit CYP2D6, one of the major isoenzymes involved in the metabolism of xenobiotics.

METHODS: Studies on the CYP isoenzymes involved in the O-demethylation of DOI, DOB, MDOB, and TMA-2 were performed with nine individual cDNA expressed CYPs (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) using microsomes of baculovirus infected insect cells as enzyme sources (details in Staack et al., Biochem. Pharmacol. 67, 235, 2004). Incubations were carried out over 30 min. They were started by adding microsomes to the incubation mixtures and terminated by protein precipitation with acetonitrile. Then, the supernatants were analyzed by LC-MS. The inhibition studies with CYP2D6 were performed by 10 min incubations and LC-MS analysis in a similar way as mentioned above. Dextromethorphan was used as CYP2D6 specific substrate (1, 2, 5, 10, 20, 50, 150, 500, 750, 1000, 1250, 1500 µM) and each of the studied drugs (250, 125, 25 µM) as well as the known CYP2D6 inhibitors fluoxetine (125 µM) and quinidine (25 nM) as inhibitors. The inhibition constants (K_i) were estimated from the K_m values of dextromethorphan as obtained in presence and absence of the different inhibitors.

RESULTS: CYP2D6 was found to be the only isoenzyme involved in the O-demethylation of the studied designer drugs, but the rate of formation was low. Besides being substrates of this isoenzyme, DOI, DOB, MDOB, and TMA-2 proved to be competitive inhibitors of CYP2D6 with the K_i values of 7.1 µM, 94 µM, 13.3 µM, and 308 µM, respectively. The K_i values for quinidine and fluoxetine were 9.2 nM and 8.2 µM, respectively.

CONCLUSIONS: The exclusive metabolism of the studied designer drugs by CYP2D6 may result in considerable variations in hepatic drug elimination due to CYP2D6 poor or ultra rapid metabolism. Furthermore, different pharmacokinetics of other CYP2D6 substrates, e.g. many pharmaceuticals or designer drugs, may occur when co-administered with DOI or MDOB the K_i values of which were similar to the known potent CYP2D6 inhibitor fluoxetine. These changes of pharmacokinetics can result in higher plasma levels and even intoxications.

Involvement of human hepatic cytochrome P450 isozymes in the N-dealkylation of MDMA, MDEA, and MBDB enantiomers

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AIMS: The amphetamine-like designer drugs 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxy-ethylamphetamine (MDEA), and *N*-methyl-benzodioxolylbutanamine (MBDB) are chiral substances. Besides the demethylenation,

another main metabolic step is the N-dealkylation to 3,4-methylenedioxyamphetamine (MDA) in the cases of MDMA and MDEA and to benzodioxolylbutanamine (BDB) in the case of MBDB. The involvement of cytochrome P450 (CYP) isozymes in this metabolic step has been studied by inhibition assays with human liver microsomes and, in part, with heterlogously expressed CYP isozymes. However, a comprehensive study on the involvement of all relevant human CYPs has not been published yet. In addition, the chirality of these drugs was not considered in these *in-vitro* studies, although their *in-vivo* metabolism is known to be enantioselective in humans. The aim of the present work was to study the contribution of relevant human CYP isozymes in the *N*-dealkylation of MDMA, MDEA and MBDB enantiomers.

METHODS: Activity screenings for general involvement were performed with nine individual cDNA expressed CYPs (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) using microsomes of baculovirus infected insect cells. Kinetic profiles were established of cDNA expressed CYPs and of pooled human liver microsomes (pHLM). From the acquired data, percentages of net clearance of the specific CYPs were calculated using the relative activity factor (RAF) approach. Incubations were started by adding ice-cold microsomes to the incubation mixtures and terminated with 60% (v/v) aqueous perchloric acid. After adding the internal standard (MDA-d_5 or BDB d_2) the mixtures were centrifuged and 50 µL of the supernatants were transferred into a 1.5 mL reaction cap. The analyte enantiomers were then derivatized to the corresponding diastereomers with heptafluorobutyrylprolyl chloride (HFBPCl) as described earlier, extracted into cyclohe–xane, separated by gas chromatography (HP 5MS, 30 m) and detected by negative ion chemical ionization mass spectrometry (NICI MS). For details see: F.T. Peters et al. (2007) Clin. Chem. [Epub ahead of print].

RESULTS: In the initial activity screening, only CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were found to be capable to catalyze the dealkylation of the MDEA, MDMA, and MBDB enantiomers. There were no principal differences in CYPs involved in the dealkylation of corresponding enantiomer pairs. The highest percentages of net clearance of *R*-MDMA (54%), S-MDMA (52%), *R*-MBDB (99%), and S-MBDB (95%), as calculated from the enzyme kinetics data, were obtained for CYP2B6. In the case of *R*-MDEA (75%) and S-MDEA (80%), the isozyme with the highest contribution to net clearance was CYP3A4. Marked enantioselectivity was observed for dealkylation by CYP2C19 with a preference for the S-enantiomers of all three substrates. In addition, CYP2D6 showed a marked preference for S-MDMA. None of the other isozymes showed major preferences for certain enantiomers.

CONCLUSIONS: It could be shown that the CYP isozyme mainly responsible for *N*-dealkylation of MDMA and MBDB (CYP2B6) is not the same as in the case of MDEA (CYP3A4) suggesting that the *N* substituent is critical for isozyme selectivity. The enantioselectivity of CYP2C19 and CYP2D6 for the S-MDMA N-dealkylation might in part explain why plasma concentrations of S-MDA are generally higher than those of R-MDA after ingestion of MDMA.

Synthesis and pharmacological testing of (R)- and (S)-temazepam glucuronides and determination of phase ii metabolism of (R)- and (S)-temazepam

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AIMS: Temazepam is mainly metabolized by glucuronidation which is the predominant pathway of phase II metabolism. D-glucuronic acid couples with the C3-hydroxy-group of racemic temazepam to form diastereomeric glucuronides. The aim of this project was the determination of enzyme kinetic data and inhibition studies of (R)- and (S)-temazepam, and the application of a novel biosensor to resolve the neuronal activity of their phase II conjugates. Therefore it was necessary to synthesize the diastereomeric pure glucuronides.

METHODS: An enzyme-assisted synthesis was developed by using swine liver microsomes, UDPGA as cofactor and racemic temazepam. A novel biosensor based on neocortical rat neurons, cultivated on a planar microelectrode array (MEA), has been used for the pharmacological testing. Determination of the pharmacokinetic drug interactions during phase II metabolism with morphine and codeine was possible by developing an in vitro UGT assay with human liver microsomes (HLMs). All commercially available recombinant UGT isoforms were screened for R- and S-temazepam glucuronidation activities.

RESULTS: By employing ESI-MS on the LTQ Orbitrap mass analyzer it was possible to determine the elemental compositions of the temazepam glucuronides with a mass accuracy < 1 ppm in comparison to the theoretical values. ^{1}H-NMR-spectroscopy supports the LC-MS/MS results. Additionally the proton of the 3-carbon showed different chemical shifts for the R- and S- glucuronide. Pharmacological tests demonstrated that the cell potential of the neurons was not significantly affected by the glucuronides. K_m and V_{max} values have been evaluated for both enantiomeres of temazepam. Experiments showed that the K_m value for S-temazepam (82.9 + -7.7 µmol) was significant lower than for R-temazepam (370.2 + -9.6 µmol). Inhibition studies

demonstrated that the two enantiomers of temazepam are affected unequal by the used inhibitors. Results showed that the glucuronidation of S-temazepam is more inhibited compared to R-temazepam. The glucuronidation of R-temazepam is mainly catalyzed by UGT2B7. The isoforms 2B7 and 2B15 are involved in the glucuronidation of S-temazepam but only in a minor fashion. The predominant glucuronidating isoform of S-temazepam could not be identified by screening UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17.

CONCLUSIONS: Temazepam glucuronides have been synthesized and fully characterized by LC-MS/MS and NMR-spectroscopy. It could be experimentally demonstrated at the cellular level that the temazepam glucuronides did not show any significant pharmacological effect on neurons by using the employed biosensor. Inhibition studies with morphine and codeine showed that the opiates inhibit the glucuronidation of both enantiomeres of temazepam unequally. Of the 12 different UGTs evaluated, only UGT2B7 exhibited significant R-temazepam glucuronidation. UGT2B7 and UGT2B15 showed minor activity for S-temazepam glucuronidation. All of the remaining UGTs demonstrated no measurable activities. (Supported by the Koeln Fortune Program / Faculty of Medicine, University of Cologne, Grants. 51/2005 and 68/2006.)

Isolation and purification of the designer drug metabolite *O*-Demethyl-*N* (1 phenyl-cyclohexyl)-2 methoxyethanamine (*O*-Demethyl-PCMEA) biotechnologically synthesized using a new fission yeast strain expressing human CYP2B6

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AIMS: Reference standards of drug metabolites are needed for their structural confirmation and pharmacologic/toxicologic characterization, but such metabolite standards are often not commercially available. Recently, biotechnological synthesis of such metabolites using human CYP2D6 heterologously expressed in fission yeast *Schizosaccharomyces pombe* was successfully used for synthesis of the designer drug metabolite 4' hydroxymethyl-alpha-pyrrolidinobutyrophenone (FT Peters et al., Biochem Pharmacol, submitted). The aim of the present study was to apply this new approach to synthesize the *O*-demethyl metabolite of the designer drug *N* (1 phenylcyclohexyl)-2 methoxyethanamine (PCMEA) using the new fission yeast strain CAD65 expressing human CYP2B6. This metabolite is structurally identical with the *O*-deethyl metabolite of the related designer drug *N* (1 phenylcyclohexyl)-2 ethoxyethanamine (PCEEA).

METHODS: For synthesis of *O*-demethyl-PCMEA, 67 mg of PCMEA·HCl were fermented with 1 L of CAD65 culture (2.4 x 10^8 cells/mL, 0.1 mM phosphate buffer pH 8, 30°C, 800 rpm, 1.5 L air/min, 96 h). After centrifugation, the supernatant was brought to pH 4 with glacial acetic acid and subjected to solid-phase extraction (SPE; Varian Bond Elut SCX HF, g, 20 ml). The eluate was evaporated to dryness and reconstituted in 3.5 mL HPLC solvent. Aliquots (250 µL) were separated by semi-preparative HPLC [Merck LiChrospher® RP select column, 250 x 25 mm, 5 µm; 50 mmol/L ammonium formate buffer (pH 3.5)/acetonitrile (80:20 v/v), 5 mL/min; UV detection at 263 nm]. The eluent fractions corresponding to the metabolite were collected, diluted with water (1:4 v/v) and subjected to SPE as described above. From the eluate, *O*-demethyl PCMEA was isolated and analyzed by GC-MS.

RESULTS: Under the given conditions, PCMEA was extensively but not completely metabolized by the heterologously expressed CYP2B6 enzymes. SPE proved useful for isolation of *O*-demethyl-PCMEA and the remaining parent drug from the incubation supernatants. *O*-Demethyl-PCMEA could be separated from the remaining parent drug and from matrix compounds by semi-preparative HPLC within 30 min. SPE also proved efficient for isolation of the metabolite from the collected eluent fractions. The identity of the product was confirmed by GC-MS.

CONCLUSIONS: Fission yeast strain CAD65 proved to be a viable tool for biotechnological synthesis of the designer drug metabolite *O*-demethyl-PCMEA. SPE followed by semi-preparative HPLC are useful tools for isolation and purification of the product from fermentation supernatants.

An estimate of the proportion of drug-facilitated sexual assaults in the USA

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AIMS: In recent years, reports of drugging potential sexual assault victims have increased. Several drugs, including ethanol, are popularly associated with what has come to be called drug-facilitated sexual assault

(DFSA). Other drugs are also candidates as factors in DFSA. DFSA means a perpetrator drugs a potential victim (here called DFSA 1) or a perpetrator takes sexual advantage of a victim who is impaired from drug and/or ethanol ingestion (here called DFSA 2). The true extent of DFSA is not known, and is difficult to estimate. The aim of the study was to estimate the proportion of drug facilitation of sexual assault in the U.S.

METHODS: The total of 144 sexual assault complainants (18-56 years of age, mean 26.6 years) at four clinics in four different parts of the U.S. (Scott & White Medical Center, Temple TX, Palomar-Pomerado Medical Center, Escondido CA, Hennepin County Medical Center, Minneapolis MN, and Providence Everett Medical Center, Everett WA) were recruited and asked to anonymously provide a urine specimen at the time of presentation, answer a few questions about suspected drugging, drug use, and the sexual assault incident, and to provide a second urine and a hair specimen about a week later. Urine and hair specimens were screened by immunoassay (EMIT) and GC-MS and confirmed by GC-MS for 45 drugs, including ethanol, common drugs of abuse, and common prescription and over the counter drugs pharmacologically capable of inducing sedation, amnesia, or impairment of judgment. Analytical test results along with subjects' statements were used to estimate the proportion of subjects and the proportion of all complainants in the same time period who were victims of DFSA 1 or DFSA 2, or whose cases were unknown but possible DFSA.

RESULTS: Between 2 and 6 percent of subject cases, corresponding to between less than 1 and 11 percent of all complainants, were characterized as DFSA 1 at the four sites. Overall, 4 percent of 144 total subject cases and less than one percent of 859 total complainants were DFSA 1. The DFSA 2 proportions were much higher: between 29 and 47 percent of subjects at four sites, and 33 percent overall; and between 5 and 39 percent of complainants at the four sites, and 6 percent overall. Eight cases overall (about 1%) were classified as unknown.

CONCLUSIONS: Characterization of a case as DFSA 1 or DFSA 2 depended on the nature and quantity of drugs found in urine, on the elapsed time between incident and presentation to a clinic, and on a complainant's statements. Sexual assault complainants in the present sample under-reported their drug use.

Application of liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization as a screening method for forty-two date-rape drugs

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AIMS: The phenomenon of drug-facilitated crimes (sexual assaults, robbery) is common in many countries. Because of wide variety of substances used as date-rape drugs, their low concentration in blood and the long delay between the alleged crime and clinical examination, analysis of biological fluids collected from victims of rapes for presence of these drugs was rare up to now. The aim of this study was to develop and apply a LC-APCI-MS screening procedure for date-rape drugs in blood.

METHODS: Target analytes were isolated using liquid-liquid extraction using MDMA-d5, estazolam-d5 and clonazepam-d4 as internal standards. Two 0.5-mL blood samples were extracted separately from acidic (pH 2) and alkaline (pH 9) media using diethyl ether and ethyl acetate. Both extracts were mixed together by sequent evaporation in one vial and than reconstituted with 200 µl of mobile phase (1:1, v/v). Analyses were carried out using an Agilent LC/MS operating in APCI mode. Separation was performed on a LiChroCART 125 x 4 column with Purospher RP-18e packing using gradient elution of 0.1% (v/v) formic acid in water and acetonitrile. Detection of all compounds was based on pseudomolecular ions that were monitored in 6 groups up to 19 ions in each group. Compounds identified in this manner were further identified by full mass spectra. The drugs were quantified in the SIM mode using calibration curves.

RESULTS: The LC-APCI-MS method allowed for the simultaneous screening, detection and quantification of forty-two compounds (arranged by retention times): morphine, codeine, clonidine, amphetamine, scopolamine, MDA, methamphetamine, PMA, MDMA, zopiclone, lidocaine, ketamine, ibuprofen, cocaine, zolpidem, phencyclidine, clozapine, meprobamate, midazolam, fentanyl, buprenorphine, diphenhydramine, doxepin, haloperidol, desipramine, imipramine, hydroxyzine, nortryptyline, amitriptyline, fluoxetine, trimipramine, oxazepam, methadone, sertraline, lorazepam, alprazolam, clonazepam, Δ^9-THC, flunitrazepam, temazepam, promethazine and diazepam. No interfering peaks were observed in the extracts of eight blank blood samples. Twenty potentially interfering compounds were individually spiked into low quality in-house control samples. All controls quantified within ± 20% of target and showed no interferences with analytes or internal standards. The LODs with a S/N≥3 were determined between 0.1 to 20 ng/mL in the SIM mode. The LOQs corresponded to the lowest calibrator concentrations with S/N≥10. The assay was linear from sub-therapeutic (0.5 - 1.0 ng/mL, 22 compounds) or low therapeutic concentrations (2 - 10 ng/mL, 14 compounds) up to 1 µg/mL (42 compounds). Linear regression correlation coefficients of the 9-point calibration curves (n = 5) were ≥ 0.990. Accuracy of the method was verified in the Qualitative Screening Analysis Program of the International Proficiency Testing Scheme with clonidine assigned concentration of 50 ng/mL. Reconstituted extracts were stable for a period of more than 24 h at room temperature or for 3 days at –20°C. The procedure can be easily expanded for more substances.

CONCLUSIONS: The LC-APCI-MS procedure was successfully applied to the analysis of authentic blood samples collected from victims of rapes in routine casework.

Stable isotopes (∂^{13}C): a proposed means of identifying the source of gamma-hydroxybutyric acid (GHB)

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AIMS: GHB is produced naturally in the human body and is also a Class C controlled substance under the Misuse of Drugs Act 1971. It is notorious because of its association with drug facilitated sexual assaults (DFSA). Studies have indicated that large variations in urinary and blood concentrations of endogenous GHB occur across population groups.[1] At present the recognised cut off values of 10 µg/mL in urine and 5 µg/mL in blood may only provide reliable evidence if the sample was taken <12 hours after ingestion because GHB is rapidly metabolised and excreted from the body.[2]
A recent study, using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), found a significant difference (> 13.5%) in the values of ∂^{13}C found in endogenous GHB in five postmortem blood samples (range: 13.8 - 86.3 µg/mL) compared to synthetically produced GHB with the implication that stable isotope measurements could significantly increase time frames of detection in reported DFSA.[3] The aim of this study is to determine the ∂^{13}C values of GHB at concentrations below the recognised cut off values in urine samples.

METHODS: GHB can be derivatised or converted to gamma-butyrolactone (GBL) for GC analysis. We have derivatised GHB using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (linear response: 0.05 - 25 µg/mL), and converted GHB to GBL (linear response: 0.5 - 50 µg/mL) based upon on a method developed 'in-house' by the Forensic Science Service Ltd.

RESULTS AND CONCLUSIONS: Preliminary ∂^{13}C (%) values for synthetic GBL at 50 µg/mL (mean -26.5%; σ_{n-1} 0.06; n = 3) and GHB-TMS derivatives at 200 µg/mL (mean -34.2%; σ_{n-1} 0.21; n = 3) have been obtained using a Thermo Finnigan MAT 253 IR-MS coupled to a Trace Ultra GC/Combustion III Interface. However, we found that extraction of GHB from urine using solid phase extraction (CLEAN SCREEN® GHB and Oasis® MAX cartridges) did not remove interfering compounds sufficiently to be able to precisely determine ∂^{13}C values at levels less than 10 µg/mL. Therefore, we propose synthesising an immunising antigen to make an antibody to specifically target GHB using immunoaffinity column extraction.

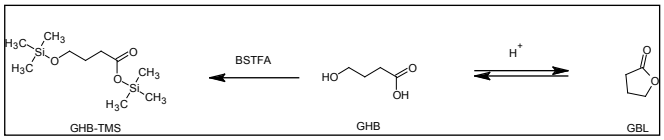

Figure 1

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Stability of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydro–cannabinol (11-OH-THC), and 11-nor-Δ^9-tetrahy–drocannabinol-9-carboxylic Acid (THCCOOH) in whole blood

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AIMS: Studies on cannabinoid stability in whole blood suggest that poor recovery of THC, 11-OH-THC and THCCOOH may result from binding to matrix components and container surfaces. Only one study has evaluated cannabinoid stability in whole blood stored in plastic tubes and none have reported changes to methodology attempting to improve recovery. Here, we report on cannabinoid recovery in whole blood samples stored in plastic at -20ºC, 4ºC and room temperature and methodological changes made in attempt to improve recovery from stored samples.

METHODS: Clinical study participants' whole blood specimens are typically collected in Vacutainer® tubes containing anticoagulant, transferred to polypropylene cryotubes and stored at -20ºC until analysis. To approximate these conditions, blank whole blood pools (15 mL, NIH blood bank) were fortified at 0.35, 1, 2, 5, 10, 20, 30 or 60 ng/mL, aliquotted (1.2 mL), stored at -20ºC, 4ºC or room temp and analyzed in triplicate on days 1 (baseline), 3, 7 and 14. Calibrators (0.125 – 100 ng/mL) and quality control samples (0.35, 2, 20, 30, 60 and 90 ng/mL) were prepared by fortifying 1 mL whole blood with THC, 11-OH-THC and THCCOOH and d3 internal standards for each. Specimens were

precipitated with 3 mL cold acetonitrile, extracted using Clean Screen® ZSTHC020 columns (United Chemical Technologies, Bristol, PA), and derivatized with BSTFA + 1% TMCS. Extracts were injected on an Agilent 6890 GC/5973MSD system (operated in EI/SIM mode). Additionally, changes to the precipitation and extraction were evaluated to determine whether recovery of analytes could be regained.

RESULTS: Two calibration curves (low, 0.125 – 25 and high, 25 – 100 ng/mL) were constructed with r^2 always > 0.99. Limits of quantification (LOQ) were 0.25 ng/mL for THC and THCCOOH and 0.5 ng/mL for 11-OH-THC. Intra- and inter-assay imprecision (%CV) was < 7% and < 9% respectively. Recovery of analytes was 85 – 104%. No chromatographic interference was detected from 20 over-the-counter, prescription and illicit drugs. After 14 d storage at -20ºC, percent recoveries (± SD) of THC, 11-OH-THC and THCCOOH (n=24), relative to baseline concentrations were 22.6 ± 13.2%, 46.7 ± 12.8% and 76.9 ± 9.9%, respectively. After 14 d storage at room temp, recoveries relative to baseline were 67.9 ± 9.0%, 69.9 ± 8.6% and 75.5 ± 5.8% (n=12). Recovery of cannabinoids from whole blood pools stored at 4ºC for 14 d was > 90% for all analytes. Methodological changes evaluated to improve analyte recovery were unsuccessful.

CONCLUSIONS: The analytical method reliably quantifies cannabinoids in freshly fortified whole blood. Data suggest reliable quantification of cannabinoids in freshly drawn authentic whole blood stored in polypropylene tubes for two weeks at 4ºC, however quantification may be low after storage at -20ºC due to significantly decreased analyte recovery. Supported by the Intramural Research Program, NIH, National Institute on Drug Abuse.

Negative-ion chemical ionization tandem mass spectrometry for fast gas chromatography analysis of cannabinoids in whole blood

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AIMS: Cannabis is considered to be the most widely abused illicit drug in Europe. Indeed, statistical information shows that 30% of the under-forties age group has already consumed this drug. Such large consumption levels necessitate fast, sensitive, and reliable methods of analysis to be devised by forensic laboratories to assist the police for investigation purposes. In humans, tetrahydrocannabinol (THC) is extensively metabolized in its two main metabolites: 11-hydroxy-tetrahydrocannabinol (THCOH) and 11-nor-tetrahydrocannabinol-9-carboxylic acid (THCCOOH). All three compounds are detected in blood and the quantification of THC is absolutely necessary in cases involving driving under the influence of drugs. Knowledge of the two metabolite concentrations becomes interesting with the application of mathematical models, which can predict the time when marijuana was consumed and also in estimating the user's driving capacity.

The purpose of our work was first, to show the power of negative ion chemical ionization (NICI) coupled with tandem mass spectrometry (NICI-MS/MS) in analyses of toxicological compounds, and second, to develop and establish the validity of a routinely applicable method that allows quantification of THC, THCOH and THCCOOH by decreasing analysis time.

METHODS: The cannabinoids were extracted from 500 µL of whole blood by a simple liquid-liquid extraction and then derivatized by using trifluoroacetic anhydride (TFAA) and hexafluoro-2-propanol (HFIP) as fluorinated agents. Mass spectrometric detection of the analytes was performed in the selected reaction monitoring mode on a triple quadrupole instrument after negative-ion chemical ionization. The following quantitation transitions were used: 410.3 > 313.3 for THC, 422.3 > 361.2 for THCCOOH and 409.2 > 339.2 for THCOH.

RESULTS: The assay was found to be linear in the concentration range of 0.5 - 20 ng/mL for THC and THCOH, and of 2.5 - 100 ng/mL for THCCOOH. The coefficients of determination (R^2) obtained for the three cannabinoids were above 0.9975 and the slope values were between 0.9926 and 1.023. Repeatability and intermediate precision were found less than 12% for all concentrations tested.

Under standard chromatographic conditions the run cycle time would have been 15 minutes. By using fast chromatographic separation conditions, the assay analysis time could be reduced to 5 minutes, without compromising the chromatographic resolution.

Our developed procedure was also used to determine the concentration levels off more than a hundred real forensic cases. Twenty-five of them will be presented with the calculation of the time prediction of marijuana use.

CONCLUSIONS: The NCI-MS/MS constitutes a true force for toxicological analysis. With its properties of soft ionization, it offers a better selectivity than electronic impact (EI) and a higher sensibility than both EI and positive ion chemical ionization (PICI), allowing use of a simpler sample pre-treatment.

Automated extraction of carboxy THC from urine on an ASPEC XL4™ Solid-Phase Extraction system without the use of SPE cartridges

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AIMS: Procedures for the extraction of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (carboxy THC) from urine by liquid-liquid extraction and solid-phase

extraction (SPE) methods have been well documented and employed worldwide. Automated SPE method for extracting carboxy THC from urine is now popular, due to its better reproducibility, increased throughput, and reduction in labor costs. However the cost of SPE cartridges is significant, especially for laboratories like ours dealing with large volume of specimens. The aim of this study was to develop an alternative automated method for extracting carboxy THC from urine without the use of SPE cartridges.

METHODS: An automated extraction protocol for extracting carboxy THC from urine was developed on the ASPEC XL4™ Solid-Phase Extraction System, employing liquid-liquid extraction principle. The process involves an initial clean-up solvent extraction step of hydrolyzed urine at basic condition (pH > 10) to remove neutral and basic interfering substances and a final solvent extraction step at acidic condition (pH < 2) for carboxy THC. Extraction is achieved by programming the System utilizing its liquid handling functions such as dispensing, mixing, and loading. The collected fractions are evaporated and derivatized with PFPA/PFPOH. The derivatives, after drying, are reconstituted in hexane for GC-MS analysis.

RESULTS: The extraction efficiency of the method (84%) was comparable to that reached with manual liquid-liquid extraction (88%) and with automated SPE (84%) on ASPEC XL system (Langen et al. 2000. *J Anal Toxicol* 24: 433-437). Excellent data concordance ($R^2 > 0.995$) was found for two patient specimen sets (n = 52) using this method and the manual liquid-liquid extraction method. The limit of detection, limit of quantitation, and the upper limit of linearity of the developed method were found at 1, 2, and 1500 ng/mL respectively. There was no detectable carry over after 10,000 ng/mL analyte. For a batch of 76 samples, the process uses 450 mL hexane/ethyl acetate (5:1) as extracting solvent and 1 L 30% methanol in water as rinsing solvent and takes 5 hrs to complete.

CONCLUSIONS: The method is comparable to both manual liquid-liquid extraction and automated SPE methods. It removes the costly SPE cartridge item from the automation process. It also removes the very demanding manual handling task of capping SPE cartridges for the ASPEC XL4™ system. The developed method is proved to be a simple, speedy and economical alternative to the currently popular automated SPE method in drug analysis of urinary carboxy THC.

A novel approach to detecting mouth alcohol

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While alcohol in the mouth exists whenever there is alcohol in the blood, the term “Mouth Alcohol” which is commonly referred to with regard to breath alcohol concentration analysis refers to a condition where the concentration of alcohol in the mouth and/or upper respiratory tract is higher than the alcohol concentration in the end expired breath. Mouth Alcohol, if present in high enough concentrations, can falsely bias the accurate measurement of end-expiratory breath alcohol.

For purposes of practical testing for breath alcohol concentrations in DUI enforcement, it is important to either utilize a testing procedure that reduces the likelihood of the Mouth Alcohol effect, or identify when Mouth Alcohol is present so that testing can be aborted for the period of time while the condition exists.

Traditionally utilized Mouth Alcohol detection algorithms identify mouth alcohol by determining if a higher alcohol concentration existed at the beginning of the expired breath sample than at end of the expiration. A breath sample where no Mouth Alcohol exists will produce an ever increasing ethanol concentration, albeit the increase is at a decreasing rate over time.

The traditional mouth alcohol waveform is influenced by a number of factors.

1) The dead space volume of the instrument.
2) The flow rate of the sample.
3) The volume of the sample.
4) The difference in concentration between the alcohol concentration in the mouth versus what is in the end expiratory breath.

This talk will present data that demonstrates how long upper respiratory alcohol concentrations remain higher than end expiratory alcohol concentrations.

This talk will discuss a novel approach to determining if Mouth Alcohol exists in expiratory breath using CO2 concentrations from the breath as a reference against which the alcohol concentration can be compared. Since the nature of the O2/CO2 transfer between the blood and deep lung breath is a similar process to the EtOH transfer from the blood to breath, it stands to reason that the CO2 concentration and EtOH concentrations should both be at their peak concentration in the alveolar breath. The approach which will be discussed assumes that if the EtOH concentration reaches a plateau or peak prior to the CO2 concentration reaching a peak or plateau, there is an indication that mouth alcohol exists. Data from tests demonstrating the technique, a discussion about its advantage over existing techniques and a review of the techniques current limitations will all be addressed in this discussion.

ROSITA II: review of oral fluid testing field trials

ROSITA II: revue des essais sur les dosages dans la salive

ROSITA II Project: evaluation of point of collection (POC) oral fluid drug testing devices at four sites in the U.S.A. and six sites in Europe (includes 5 presentations)

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(5) Ghent University, Gent, Belgium

Presenters and Titles of Individual Presentations:
J. Michael Walsh, Ph.D., Moderator

1) Jayne Thatcher, "Evaluation of On-Site Saliva Drug Testing Devices in Washington State"
2) Laura Liddicoat, "Evaluation of On-Site Saliva Drug Testing Devices in Wisconsin"
3) Leo Cangianelli, "Evaluation of On-Site Saliva Drug Testing Devices in Hillsborough County FL"
4) Denny Crouch, "Evaluation of On-Site Saliva Drug Testing Devices in Salt Lake City, Utah"
5) Alain Verstraete, "Review of EU Partner sites participating in Rosita II, Belgium, Finland, France, Germany, Norway, and Spain"

This collaborative US/EU international effort was carried out from 2003 – 2006 to evaluate the feasibility of using POC oral fluid drug testing devices in the enforcement of drugged driving laws. The ROSITA II project was conducted in major cities in the US and Western Europe by teams of scientists working in collaboration with local police. This session will present specific data from the U.S. sites and a comparison summary of the European partner sites. Six POC oral fluid drug testing devices were evaluated in the U.S. field study [Drugwipe® (Securetec), OralLab® (Varian), Oratect® (Branan Medical), RapiScan® (Cozart), SalivaScreen® (UltiMed) and Uplink® (Orasure Technologies). The devices were randomly assigned to the partner sites.

Standardized training of all research and police teams was conducted prior to the field evaluation to ensure that the protocol was carried out uniformly across sites. Standard police measures were used to identify drivers suspected to be under the influence of drugs. Two oral fluid specimens were collected from each DUI suspect. One specimen was used with the POC device, and the other was shipped to a reference laboratory for comparison analysis. Depending on specific State laws, blood and or urine specimens were also collected from the suspect.

The evaluation focused on the performance of the devices [specificity, sensitivity, accuracy] as well as the user friendliness for police in field operations. In general, most of the devices detected methamphetamine, amphetamines, and opiates well and detected those drugs in the range of the cutoff concentrations proposed by SAMHSA. The ability to accurately and reliably detect cocaine was device dependent. None of the devices performed well in detecting marijuana use. This remains a key concern with the use of POC OF devices because marijuana is the most commonly abused illegal drug and of primary concern for drugged driving cases. Of the six devices evaluated in the U.S field study only the Drugwipe was considered acceptable for roadside testing based on its ease of use and the willingness of the donors to provide specimens. However, the Drugwipe device was not sufficiently sensitive to detect the two primary drugs of abuse commonly detected in DUID arrests, i.e. marijuana and cocaine.

Prevalence of drug in driving populations

Prévalence des médicaments chez les conducteurs

Erik MP Widmark – Bridged the gap between forensic toxicology and alcohol and traffic safety research

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The name of Erik MP Widmark is widely recognized by both forensic toxicologists and those working in the field of alcohol and traffic safety research. Indeed, ICADTS created the Widmark award in 1965 to honor those scientists making seminal and sustained contributions to the field of alcohol, drugs and traffic safety.

Erik Widmark was born in 1889 in the Swedish town of Helsingborg. He studied medicine at the University of Lund and defended a thesis in 1917, which dealt with the determination of acetone in blood, breath and urine and the pharmacokinetics of this endogenous volatile substance. Widmark applied for and was appointed a full professor in Medicinal and Physiological chemistry at the University of Lund in 1920 at the age of 31 years.

The bulk of Widmark's research focused on the disposition and fate in the body of alcohol and the factors influencing the blood-alcohol concentration (BAC) attained after drinking. In 1922 he published a highly practical micro-diffusion method, which permitted

quantitative analysis of ethanol in capillary fingertip blood samples. Using this analytical method, Widmark established the relationship between clinical signs and symptoms of drunkenness and the prevailing BAC. Studies such as these proved extremely useful when the Swedish government in 1941 introduced a punishable BAC limit (per se law) for driving (0.8 mg/g or ~0.08 g%). The statutory alcohol limit for driving in Sweden today is much lower being set at 0.2 mg/g (~0.02 g%).

The principles of clinical pharmacokinetics were established by Widmark long before the word was coined by the German scientist Dost in 1953. Among other things, Widmark traced the time-course of alcohol in blood after drinking on an empty stomach or together with a meal. This research led to development of the "Widmark equation" including the factors ß and rho, arguably the most widely used calculation in the forensic sciences and legal medicine. This fundamental research was published in 1932 in a monograph written in German and translated into English 50 years later, giving testimony to the longevity and usefulness of its contents. Other articles by Widmark were written in Swedish, English and French although the vast majority was in German language journals, e.g. Biochemische Zeitschrift.

The role of nutrition and the importance of eating a balanced diet including vitamins and minerals also interested Widmark. He was the first to demonstrate the dangers of eating burnt foodstuffs and the risk of developing certain malignant tumors. This article was published in the British journal NATURE long before the Ames test for the mutagenic potential of chemicals was developed. Widmark's last alcohol research paper dealt with use of animal models (dogs) to study aspects of tolerance and the development of organ and tissue damage caused by chronic drinking. Widmark enjoyed a productive academic career in the medical sciences especially physiological alcohol research and gained international recognition for his contributions. He received various awards and medals for his work and was elected into the Royal Swedish Academy of Sciences in 1938. Widmark's health began to deteriorate in later years and he died on the 30th April 1945 aged just 55 year. On the death certificate hypertension and coronary artery disease were mentioned as contributing factors.

Erik MP Widmark made seminal contributions to the field alcohol and traffic safety research during the first half of the last century. His work on the pharmacokinetics of ethanol and acetone remain classics of the literature. His micro-method for blood-alcohol analysis was pivotal in creating punishable BAC limits for driving in several European countries. The name of Erik MP Widmark is ensured and his legacy enhanced by creation of the prestigious Widmark award.

Frequency of illegal drugs and medicines in a Norwegian road-side survey

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BACKGROUND: Traffic safety is one of the main goals of the World Health Organization. One of the major research areas of the Norwegian Public Health Institute is the relation between abuse of drugs and traffic safety in Norway. The aim of this study was to investigate the use of alcohol, illegal drugs and psychoactive medicines among ordinary drivers. The project was performed in collaboration with the Institute of Transport Economics with the assistance from the Mobile Police Force.

METHOD: To obtain a high percentage of participation of the drivers, a saliva sample was selected as biological specimen for analyses of drugs and medicines from at least 10,000 randomly selected drivers during a 12-month period in the South-East part of Norway. The drivers were informed both orally and with a leaflet before they gave an informed consent to participate anonymously in the study. The saliva samples were analysed for the use of morphine, heroin, codeine, methadone, buprenorphine, amphetamine, metamphetamine, MDMA, MDA, MDEA, cocaine, THC, LSD, diazepam, flunitrazepam, clonazepam, nitrazepam, oxazepam, alprazolam, lorazepam, bromazepam, fenazepam, zopiclone, zolpidem, carisoprodol and some metabolites by LC-MS/MS and alcohol by an ADH-method.

RESULTS: Close to 11,000 drivers were included in the study and 89% of all drivers asked, were willing to participate. Drivers of private cars made up some 80% of the survey samples, and vans, trucks and motorcycles made up 15%, 2% and 1%, respectively. The average age of all included drivers was 45 years. 30% of the drivers were women.

The medicines diazepam, flunitrazepam clonazepam, nitrazepam, oxazepam, alprazolam, zopiclone, zolpidem or carisoprodol was detected in saliva from about 4% of the drivers and whereas morphine, heroin, codeine, methadone, buprenorphine, amphetamine, metamphetamine, MDA, cocaine, THC was found in saliva from about 1% of the drivers. The most common medicine was the hypnoticum zopiclone, and THC was the most common illegal drug. Alcohol was detected in about 0.5% of the drivers.

CONCLUSIONS: This study shows that most Norwegian drivers are willing to deliver anonymously a saliva sample for testing of illegal drugs and medicines. During a period of 12 months about 11000 randomly selected drivers were included in a study where the frequency of drugs and medicines with a potential to

reduce driving skill was investigated using saliva. About 4% of the drivers had at least one medicine in the body and about 1% of the drivers had at least one illegal drug in the body. The frequency of medicines among the drivers is comparable to the frequency of the sale statistics of these medicines.
This study was financed by the Norwegian Directorate for Health and Social Affairs and the Norwegian Ministry of Transport and Communication.

Searching the scholarly literature for articles concerning alcohol, drugs and traffic safety

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A literature search for articles on concepts in the alcohol, drugs, and traffic safety (ADTS) field may seem to be a straightforward process. However, conducting a focused and comprehensive search can be time consuming and difficult even if the searcher is a librarian with specialized training and experience. Many scientists and practitioners are unaware of their own lack of knowledge about the information sources and searching mechanisms they use. This may lead them to conduct their own searches when it isn't convenient to consult a librarian. This report will describe several issues that contribute to the difficulty of conducting a productive literature, the results of a series of investigations designed to quantify the problems, and efforts that are underway to resolve some of the issues and improve the availability of ADTS research articles.

Researchers and other professionals from at least 30 distinct disciplines publish reports that are relevant to ADTS. Among these are anthropology, chemistry, codes and standards development, consumer product testing and safety, demography, dentistry, economics, education, engineering specialties, ergonomics, industrial design, interior design, law, management and administration, marketing, media studies, medicine, nursing, occupational safety and hygiene, pharmacology, physiology, political science and policy, psychology, public health, public safety, social work, sociology, sports and kinematics, toxicology, transportation safety, urban planning, and other fields. These reports are published in hundreds of different journals. No single literature database includes all relevant journals. Indeed, a search of the contents of all relevant journals will require queries of several literature databases. An article on the same concept is likely to be indexed with different terms in each database. This is because the contents of each database are assigned index terms by professional indexers who must only assign the index terms from the specific vocabulary of the thesaurus developed for the particular database and must assign the terms according to a selection protocol relevant to the purpose of the specific database and its target users (i.e. behavioral issues for PsycINFO, bio-medicine for PubMed, engineering issues for EI Compendix, etc.).

Searching is further complicated by the different jargon used within each of the disciplines to describe similar concepts. This is still further complicated because different terms may be used for the same concept depending upon the English-speaking nation where the author resides. For example, we found found 18 different terms used by authors and searchers for the concept "ethanol-impaired driving" and an additional 5 terms that refer to legal BAC limits.

A tool is under development that may aid a searcher's efforts to find a list of all synonyms necessary to conduct a comprehensive search for articles on an ADTS concept. Although it remains a work in-progress, it may prove to be useful as a source of synonym terms even though it is incomplete. A draft of the *Injury Prevention and Safety Promotion Thesaurus* is available online at http://www.injurypreventionthesaurus.com.

Once complete, electronic copies will be available online at no cost. The thesaurus will be connected to the SafetyLit archive so that searches will be facilitated. SafetyLit is a free service of San Diego State University and the World Health Organization. The SafetyLit archive contains (among other things) the complete ADTS-related contents of more than 2900 scholarly journals from volume 1, issue 1.

Driving under the influence of psychoactive substances

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In 1999, Belgium introduced a law on driving under the influence of illegal drugs. In 2005 the Belgian Science Policy has taken the initiative to support a survey intended to draw up recommendations for a more efficient enforcement policy.

An exhaustive literature study on the influence of drugs on fitness to drive and a survey about the legislation and enforcement in a number of European and non-European countries were conducted. Various initiatives such as surveys among police forces, police schools, accredited laboratories and public prosecutors were taken to evaluate the application of the law in Belgium. The research team observed also targeted controls organized by the police.

The results of the study revealed the problems of the current procedure. Based on these observations, preliminary recommendations were formulated. These recommendations were discussed with national experts and adapted to be presented for comments to six international experts and a Belgian expert on penal law. The therefore used method was the Delphi-method. Finally, the preliminary recommendations were transposed into final recommendations.

The recommendations described in the report can be

subdivided in five themes. Covered topics are the lack of data, practical problems for the police, problems with regard to the prosecution, legislation and analysis of blood samples.
In Belgium there are no recent data on the number of drug-related accidents. Therefore, a toxicological analysis should be carried out on all drivers involved in a fatal accident.
The problems the police are faced with are multiple. Most important is the fact that the procedure is time-consuming and complicated and that only 18% of the officers have had the required training. The researchers propose that efforts should be made to simplify the procedure by introducing new, reliable, quicker and easier-to-use tests.
The study of the prosecution shows that the different public prosecutors give different instructions to the police. The researchers recommend that all public prosecutors work in a uniform way.
The research also reveals a number of problems with regards to legislation. These problems concern among others the possibilities to withdrawn the driving licence, inexperienced drivers and persons who are dependent on psychoactive substances.
During the research, some problems with regards to the blood sampling and the analysis of the sample were revealed and propositions were made.
In the last part of the report some suggestions with regard to awareness-raising, communication and further research are made.

Studies on drugs and driving realised in european countries: focus on cannabis and benzodiazepines

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The EMCDDA decided to look into the topic of cannabis and benzodiazepines in European drivers, to support or correct the findings of earlier studies that these were the two most prevalent substances in this particular population.
In 2006, the EMCDDA requested each of its national partners (REITOX network of national focal points) to supply information available concerning the issue of drugs and driving with a specific focus on the above mentioned substances. This presentation aims at providing an insight on the main conclusions of the prevalence studies submitted. Though a large number of prevalence studies on drivers have been carried out at national level in European countries during the last 10 to 15 years, not all included equal focus on cannabis and benzodiazepines. Furthermore, the major limitation in drawing a picture of the prevalence of drugs in drivers is the problem of comparability of results. The reasons for this include differences in sampling methodology, choice of drugs to screen, specimens collected, screening devices, and cut-off levels for forensic analysis. On a cross-national level, different legislative frameworks add further complexity.
This study of roadside surveys (random or on suspicion), hospital or accident studies, confirms that cannabis and benzodiazepines, besides alcohol, are the two psychoactive substances most prevalent among drivers in Europe, although a few exceptions can be pointed out. In Finland, Sweden and Latvia amphetamines were frequently found, opiates seemed to feature in Greece and Germany, and cocaine was the most prevalent substance in drivers in Spain. Concerning benzodiazepines, limited reports of blood concentrations showed levels both therapeutic and clearly above. Finally, polydrug use cannot be ignored.
It appears from this European overview that better national drugged-driving estimates are needed, based on reliable epidemiological studies of drivers, particularly when drug prevalence estimates among drivers do not always match those of the general population. This would then enable design of more effective policy responses for each psychoactive substance.

Investigations on driving under the influence of drugs in Vienna: experiences from 1996 to 2006

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With effect from 1994, §5 of the Austrian road traffic regulations 1960 (StV0) has legalized blood sampling for the use of chemical analysis whenever a driver is suspected to use illicit drugs. Since its amendment in 2002 the sampling of blood by an authorized physician is mandatory and the driver has to give his consent to the procedure, in case any impairment is suspected; otherwise the impairment by the use of narcotic drugs is taken for granted.
In contrast to the legal regulations of the neighbouring countries (The Federal Republic of Germany and Switzerland), where analytical detection limits are taken as evidence for impairment, Austria did not follow this procedure, as only impairment due to recent drug use can be detected, but any impairment in the course of withdrawal symptoms is not reflected by this approach. Moreover the implementation of §24 StVG in Germany in 1997 restricted the number of target analytes to 6 active substances or their metabolites, which justify an administrative offence. According to the Austrian StVO the consumption of all narcotic drugs, defined in the Single Convention and the Austrian SMG respectively, are prohibited, leading to a far larger number of target substances. After the introduction of

immunological testing of urine samples in 1994 to serve internal purposes of the Police Headquarter in Vienna, such urine tests have been applied to drivers under the influence of drugs since 1996. In case of positive results the samples were submitted to an authorized laboratory for confirmation by gas chromatography – mass spectrometry. In 1996 about 100 impaired drivers could be diagnosed. Until 1999 this number increased to about 600 positively identified cases.
Since another amendment in effect from 2002, blood sampling has become obligatory, the analysis of urine samples is possible. Since 2005 such investigations have been performed in ISO 17025 certified laboratories by means of gas chromatography – mass spectrometry, as well as by using liquid chromatography and tandem mass spectrometry.
The presented data compare the number of impaired drivers in Vienna between 1996 and 2000 on the one hand, with those between 2005 and 2006 on the other. For the first time a collective representing more than 1,000 individuals was accessible, with the chemical analysis including quantification of the substances. It turned out that most of the impaired drivers consume more than one substance only and the prevalence of Ecstasy has increased significantly. The investigations prove that the pattern of drug abuse follows their local availability.

Prevalence of drug impaired driving fatalities in Canada 2000 - 2004

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In Canada, information on fatally injured impaired drivers has been collected over the past 30 plus years. These data have been useful in monitoring the challenge of alcohol impaired driving in Canada and are presented nationally and for each provincial/territorial jurisdiction. In order to track the incidence of drug impaired driving, the Strategy to Reduce Impaired Driving (STRID) fatality database, collected by the Traffic Injury Research Foundation on behalf of Transport Canada and the Canadian Council of Motor Transport Administrators was modified to collect drug related information. The STRID alcohol database is based on a very high testing rate of alcohol among fatally injured drivers, the STRID drug database represents two models of testing. In the first, the testing rate is high, defined as above 70% while in the second model the testing rate is based on a suspicion of drug involvement and averages 35% of all eligible drivers.
This current research is important to learn more about the magnitude of drug-impaired driving at the national and jurisdictional levels. The aim of this study is to elucidate the current situation of drug impaired driving in Canada. A unique feature of the study is that it contains quantitative information on the presence of drugs among fatally injured drivers. To date, the mechanism by which various drugs might contribute to vehicle crashes is not well understood. The results of this study provide information on the testing rate for drugs among fatally injured drivers in Canada, and separately by jurisdiction. In addition, aggregate analysis will identify the frequency of drug classes. Separate breakdowns will be provided differentiating between jurisdictions with low-testing rates and those with high-testing rates.
Results are based on 9,158 fatally injured drivers and looks at rates by types of drugs by jurisdiction in Canada. The period of the study is from 2000 to 2004.

Blood drug concentrations of frequently encountered drugs in impaired and fatally injured drivers

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This presentation will review blood drug concentrations of drivers in DUID arrests, vehicular assault and homicide cases, and fatally injured drivers, and present these as a basis for assessing future blood concentrations in suspected impaired drivers. The Washington State Toxicology Laboratory compiled data for the most frequently encountered drugs in three impaired driving populations. The first was a group of drivers arrested for suspected impaired driving. There were 182 different drugs and metabolites detected in 9,772 cases from whom blood samples were submitted for drug testing. A total of 17,213 positive results. The most frequently encountered drugs, with their frequency, mean, median and range of concentrations are shown in Table 1.

Table 1*. Blood drug concentrations of 12 most frequently encountered centrally acting drugs and metabolites in impaired driving cases.*

		concentration (mg/L)*			
Drug	Frequency	Mean	Median	Range (lo)	Range (hi)
THC*	926	6,26	5	3	48
*Carboxy-THC**	3102	27,75	18	10	838
Methamphetamine	1159	0,30	0,21	0,010	9,46
Amphetamine	539	0,07	0,05	0,005	5,09
Cocaine	702	0,08	0,03	0,005	2,39
Benzoylecgonine	906	1,24	0,86	0,005	17,60
Diazepam	764	0,25	0,12	0,005	3,20
Nordiazepam	737	0,27	0,11	0,010	3,67
Morphine	676	0,05	0,03	0,001	1,29
Carisoprodol	514	4,77	3,90	0,050	25,10
Meprobamate	627	14,5	12,30	0,500	77,60
Hydrocodone	289	0,05	0,02	0,005	0,56

**All concentrations in mg/L, except where indicated (*) ng/mL*

A recent report describing the concentrations of drugs in fatally injured drivers (*Schwilke EW, Sampaio dos Santos MI, Logan BK. Changing patterns of drug and alcohol use in fatally injured drivers in Washington State. J Forensic Sci. 2006 Sep;51(5):1191-8*) found many of the same drugs with similar concentrations, including the same top three. These drivers tested positive for THC (mean 8 ng/mL, median 6ng/mL, range 2 - 32 ng/mL); Cocaine (mean 0.72 mg/L, median 0.31 mg/L, range 0.03 – 3.30mg/L); and Methamphetamine (mean 0.73 mg/L, median 0.26 mg/L, range <0.01 – 1.08 mg/L). Having a large reference population of other drug affected drivers helps to assess concentrations in future cases, where a subject may seek to minimize the degree of drug use, or the significance of the concentrations.

Recommendations for toxicological investigation of drug impaired driving

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Investigation of a suspected alcohol or drug impaired driving (DUID) case ideally contains several key elements, including a trained officer documenting observations of driving and subject behavior, and collection of a biological specimen for comprehensive toxicology testing. There currently is no common standard of practice among forensic toxicology laboratories in the United States as to which drugs should be tested for, and at what analytical cutoff. Having some uniformity of practice among laboratories would ensure that drugs most frequently associated with driving impairment were consistently evaluated, that appropriate methods were used to screen and confirm the presence of drugs, and that more accurate data were collected on the extent of drug use among drivers. A survey of United States laboratories actively involved in providing analytical support to the Drug Evaluation and Classification Program identified Marijuana, Benzodiazepines, Cocaine, Hydrocodone, Morphine/Codeine, Metham–phetamine, Carisoprodol/Meprobamate, Oxycodone, Methadone, Antidepressants, Zolpidem, PCP, Butalbital/ Barbiturates, Diphenhydramine, MDMA, Propo–xyphene, Ephedrine/Pseudoephedrine, Cycloben–zaprine, Dextromethorphan, Gamma-hydroxybutyrate, Ketamine, Phenothiazines, and Tramadol as being the most frequently encountered drugs in these cases. Based on these findings, we present recommendations as to what specific members of these drug classes should at a minimum be tested for in the investigation of suspected DUID cases. Additionally we include recommendations for analytical cutoffs for screening and confirmation of drugs in blood and urine. Adopting these guidelines would ensure that the most common drugs would be detected, that laboratories could compare epidemiological findings between jurisdictions, and that aggregate national statistics on alcohol and drug use in drivers involved in fatal injury collisions would be representative of the true rates of drug use in the driving population.

An overview of the existing drugs and driving categorisations

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One of the tasks (WP 4.1) of the DRUID research project is to review the existing classifications of drugs, known to produce driving impairment. Eventually, a consensus and synthesis will have to be drawn within this work package, as to find a uniform classification system.

An inventory of existing categorization systems was made. Eleven systems have been found, involving in between 33 to 570 different drugs/formulations. In Belgium, the categorisation of substances is done according to Wolschrijn et al. These are expert ratings that categorize in 7 classes, from no impairment to severe impairment. Over 570 molecules were evaluated. Germany applied the same categorisation system. In Italy a literature review was done for 44 molecules. The Nordic countries, Norway and Finland, apply a red warning label, when a substance is dangerous. These are marked in their list as positive. The Netherlands also apply a positive list (with a yellow warning label). All of these categorisations existed already before 1999. However, since then, new countries and substances were added. Denmark has a positive list of 70 different substances. At the moment Spain has 2 systems: one by the DGT (Dirección Gral. De Tráfico) with 3 categories, and one by the SemFYC (Sociedad Español de Medicina de Familia y Communitaria) with 4 categories. France, also has 2 systems. The first one by the CERMT (Centre d'Études et de Recherches en Médecine du Trafic) contains 4 categories, the other one, published in 2005 in the 'Journal officiel de la République Française' consists of 3 kinds of warning labels. The ICADTS system is a categorisation system which combines the lists of Belgium, France (list of 2005) and Spain (DGT). Through a questionnaire, sent to DRUID and non-DRUID partners, an assessment of the validity of the classification systems of every country is made. As in the ICADTS system, the drugs are grouped according to the ATC. This way about 9 large groups with several subgroups can be identified. In total over 700 different substances and their combinations are reviewed.

In general, substances in the different classifications are classified in the same category of impairment, although sometimes opinions differ. The mean goal is

to find a consensus for each substance and a correct and practical classification, easy to access and to use for health care professionals. All substances, available in Europe, should be evaluated according to the new standards developed. New substances should be easily classified within the new system.
As for this, WP 4 will contribute to another DRUID work package (WP 7) to help formulate guidelines and booklets.

Fatal motor vehicle collisions while gamma hydroxybutyrate (GHB)-intoxicated

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BACKGROUND AND OBJECTIVES: Gamma hydroxybutyrate (GHB) and its analogs (GHB/analogs), gamma butyrolactone (GBL) and 1,4 butanediol (BD), are drugs of abuse that were also sold as "dietary supplements" for purported health effects including bodybuilding, weight loss, and sleep. We sought to investigate GHB-associated deaths, including deaths resulting directly from GHB as well as deaths resulting from fatal accidents suffered while GHB-intoxicated, with specific focus here on fatal motor vehicle collisions (MVC's).

METHODS: We collected autopsy and toxicology reports from medical examiners and coroners in the US, UK and Canada for GHB-associated deaths from 1995-2005. Historical data was obtained from investigative summaries, ProjectGHB (an informational website, www.ProjectGHB.org) and media reports. Cases were reviewed for GHB levels, cause of death (COD), pathology, medical history, history of GHB use, and history of terminal event. Inclusion was based on GHB detected above inclusion cut-offs in at least one tissue sample; gas chromatography/mass spectrometry inclusion cut-offs were, respectively, 5 and 10 mg/L GHB in antemortem (AM) blood and urine, and 50, 20, and 7 mg/L GHB in postmortem (PM) blood, urine, and vitreous fluid. Cases ruled positive for co-intoxicants had a parent drug or active metabolite quantified in blood. Statistics were descriptive. We calculated the range of GHB blood levels and mean level ± confidence interval (CI) in deaths confirmed negative for co-intoxicants (GHB-only) resulting from GHB toxicity (with no accident) vs. GHB-only MVC deaths. Groups were compared by non-parametric testing (Kruskal-Wallis).

RESULTS: A total of 226 GHB-associated deaths were identified. Of these, 213 decedents suffered cardiopulmonary arrest and 13 were in fatal traumatic events, including 6 drownings, 1 smoke inhalation, and 6 MVC's. Of the 6 MVC's, 3 involved GHB-intoxicated DRIVERS and 3 involved GHB-intoxicated PEDESTRIANS. The DRIVERS included two women and one man, ages 29, 31, and 30 years, respectively. Two GHB-only deaths had PM blood GHB levels of 164 and 240 mg/L. Intoxicants identified in PM blood in the third death included GHB (53 mg/L), MDMA (1.07 mg/L), and EtOH (0.22 g/dL). All three deaths documented neck fractures, of which two were neck transections. One driver had a history of acute GHB ingestion. Another was seen slumped over the steering wheel prior to the collision and had a past history of dietary supplement use and a prior police stop for impaired driving, in which her blood tested negative for EtOH and unspecified drugs. The third driver struck another auto, with both drivers killed instantly, and no additional history. The three PEDESTRIANS included 3 men, 2 of ages 29 and 27 years, and one with age unknown. Two of the pedestrian deaths were GHB-only, one with PM blood GHB 100 mg/L and the other with AM blood GHB 159 mg/L. PM blood GHB in the third death was 179 mg/L, with blood also documented as positive for methamphetamine (unquantified). One pedestrian was struck when he exited a car in the middle of a highway (no additional history). The second was "struck by an automobile as he attempted to cross the highway in just underwear," with a history of acute GHB ingestion. The third was hit by a train, with no additional history known. The mean PM blood GHB level +/- CI in 64 GHB-only deaths from GHB toxicity (with no accident) was 544 +/-152 mg/L (range 18-4400 mg/L, median 347 mg/L) vs. a mean blood level of 165 +/- 56 mg/L (range 100-240, median 162 mg/L) in 4 GHB-only MVC deaths ($p = 0.012$).

CONCLUSIONS: GHB-associated MVC deaths may involve both drivers and pedestrians. Cervical spine transection may suggest GHB (or other sedative/hypnotic) toxicity, although numbers are too small for conclusions. GHB blood levels are significantly lower for those who die in MVC's vs. those who die of GHB toxicity alone.

Forensic alcohol toxicology
Alcool et toxicologie médico-légale

The uncertainty associated with Widmark's equation in forensic toxicology

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AIMS: Widmark's equation is probably the most frequent computation performed in forensic toxicology. The equation is typically employed to estimate either the number of drinks consumed or the corresponding blood or breath alcohol concentration in either legal, research or training contexts. Despite its wide use, Widmark's equation is rarely accompanied by an estimate of its uncertainty. This may arise from either a reluctance to acknowledge uncertainty in a legal context or from unfamiliarity with the necessary computations. Widmark's equation, a simple algebraic model relating alcohol consumed to volume of distribution and blood alcohol concentration, contains seven random variables, each having differing magnitudes of uncertainty. Ideally, these should be accounted for when presenting Widmark estimations.
The aim of this presentation is to present straightforward estimates of uncertainty when using Widmark's equation to determine either the number of drinks consumed or blood alcohol concentrations.

METHODS: Uncertainty estimates are presented here for Widmark calculations that rely on methods of general error propagation – a method that combines both the algebraic form of the equation along with variable uncertainty estimates. The results of general error propagation are then compared to a method developed by Widmark along with another method developed by Alha, a Finnish researcher.

RESULTS: Assuming reasonable variable and uncertainty values for a typical Widmark calculation, the error propagation method yielded N = 10.4 drinks along with an uncertainty (standard deviation) of 1.3 drinks (CV = 12.3%). Similarly, estimating the blood alcohol concentration for the same set of assumed data yielded 0.120 g/100mL along with an uncertainty of 0.0255 g/100mL (CV = 21.2%). Widmark's uncertainty method, by comparison, yielded an uncertainty of 1.6 drinks (CV = 15.4%). Similarly, Alha's method yielded an uncertainty of 0.8 drinks (CV = 8.0%).

CONCLUSIONS: The derivation of Widmark's uncertainty estimate reveals that he considered only ρ and β to be uncertain. Moreover, he failed to include their correlation in his calculations. Alha, on the other hand, also included only ρ and β as uncertain variables but did include their correlation (r = -0.58). Both Widmark and Alha's uncertainty equations are derived from the general error propagation model as well. While ρ and β are clearly the variables with the largest uncertainty, employing the method of general error propagation and including all seven variables, provides a more thorough estimate of uncertainty. Generally, Widmark estimates for the number of drinks should include a 2CV estimate of approximately 24% while the blood alcohol concentration estimate should include a 2CV estimate of approximately 42%. Including valid uncertainty estimates should enhance both the legal confidence and research credibility associated with Widmark estimations.

Rapid variation of the ethanol distribution volume - an unexpected phenomenon

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AIMS: For alleged drinking after driving (hip-flask or glove compartment defence) the German traffic law requires calculation of the ethanol intake after an incident. To this end, a minimum resorption deficit (RD) of 10% is assumed for the after-drink volume. (Resoprtion deficit is a synonym for first pass effects of alcohol metabolism; after-drink refers to the intake of an additional amount of alcohol after the offence). Proceeding from the fact that the RD is mainly based on first pass effects, this assumption holds true only up to the point whereby the ethanol-decomposing enzymes have reached their maximum turnover capacity. As a consequence, an RD is not likely to be found in cases where ethanol is consumed on top of an existing blood alcohol level. This hypothesis was tested by experiments.

METHODS: Healthy subjects (so far 14 male and 7 female) drank 0.5 g of ethanol per kilogram of body weight. After the breath alcohol concentration curve had reached its peak, ethanol was administered by infusion (mean 0.106 ± 0.010 g/Kg/h, approx. 4.5 hours) to obtain a steady state. During this phase, however no earlier than 2 hours after the first drink, the subjects drank an additional quantity of 0.25 g of ethanol per kilogram of body weight. Infusions were continued until a new equilibrium had been reached. The test procedure was controlled by breath alcohol concentration measurements (Dräger Alcotest® 7110 MK III-C) and concurrent blood sampling according to applicable forensic guidelines.

RESULTS: Individual volumes of distribution (VD) of the subjects were obtained from the ratio between the quantity of ethanol administered per time unit in steady state and the linear ethanol elimination rate, and reached expected values (Widmark "r" male 0.74 ± 0.14; female 0.59 ± 0.09). On this basis the values anticipated for the increase of the blood alcohol concentration as a result of the after-drink could be precisely established.

An unexpected phenomenon was found in most of the male subjects: the blood alcohol concentration increase as a result of the after-drink (position of the steady state without peak values) was 20% higher on average than the maximum expectation value suggested by Widmark. This phenomenon was attributable, for instance, to a reduction of the distribution volume. Assuming that the after-drink RD is negligible, the after-drink VD decreased from 0.74 to 0.63 in male subjects. Only 2 of 7 women responded in a similar manner.

CONCLUSIONS: Performing drinking tests we observed rapid changes in the distribution volume of ethanol. Short-term variations of the effective VD are attributed to vasomotor effects. Such effects should be considered under special circumstances like shock, injuries or vomiting during the resorption phase. On the other hand, it also seems to be a physiologic reaction in normal ethanol pharmacokinetics. As expected, an after-drink RD was not found.

Comparison of the urinary alcohol markers ETG, ETS and GTOL/5-HIAA in a controlled drinking experiment

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AIMS: Urinary ethyl glucuronide (EtG), ethyl sulphate (EtS) and the ratio between 5-hydroxytryptophol-glucuronide and 5-hydroxyindoleacetic acid (GTOL/5-HIAA) are used as biomarkers for alcohol relapse with longer detection times compared with measurement of ethanol itself. This controlled study determined the sensitivities and detection times of EtG, EtS and GTOL/5-HIAA in urine samples collected after a single ingestion of ethanol.

METHODS: Ten healthy male volunteers ingested 0.5 g ethanol/Kg body weight after an overnight fast. Urine collections were made before start of drinking, then at every voiding until 45 - 50 h after the start of drinking. This corresponded to approximately once every 2 h for the first 8 h after intake, thereafter at variable intervals. The total volume of each urine collection was determined. Ethanol was measured by headspace gas chromatography equipped with a flame ionization detector. EtG, EtS and the ratio GTOL/5-HIAA were determined using a liquid chromatography-mass spectrometry (LC-MS) method. The limit of detection (LOD) was for ethanol 0.005 g/L and for EtG and EtS 0.1 mg/L. All values above these levels were reported as positive results. For the ratio GTOL/5-HIAA, an administrative cut-off (15 nmol/µmol) was used and values above this level were reported as positive.

RESULTS: During the first 8 h after drinking, urinary EtG, EtS and GTOL/5-HIAA showed 100% sensitivity as alcohol markers. The median maximum concentration (C_{max}) for ethanol was 0.6 g/L (range 0.4 - 0.7), and for GTOL/5-HIAA 275 nmol/µmol (range 199 - 622). The C_{max} for EtG (median 60 mg/L, range 47 - 88) was higher than for EtS (median 21 mg/L, range 14 - 29) in all subjects. Compared with ethanol testing, the detection times for GTOL/5-HIAA were ~5 h longer and for EtG and EtS ~25 h longer. A higher fraction of the ethanol dose was excreted as EtG (median, 0.019%) compared to EtS (median, 0.011%).

CONCLUSIONS: Measurement of EtG, EtS and GTOL/5-HIAA in urine was confirmed useful as biomarkers to detect recent drinking. Positive results were obtained for some time after the ethanol has been eliminated with the longest detection times for EtG and EtS. The GTOL/5-HIAA ratio was equally sensitive in the short run, but showed a much shorter window of detection. In cases where surveillance of alcohol relapse is needed, EtG and EtS in urine are excellent alternatives to ethanol measurement.

Ethyl glucuronide in vitreous humor: a useful marker of antemortem ethanol ingestion?

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AIMS: Ethyl glucuronide (EtG) has shown to be a sensitive and specific biomarker for alcohol intake. Its detection in blood and others postmortem body fluids and tissues, barring vitreous humor (VH), has been reported as an aid in interpretation of antemortem alcohol ingestion. The aims of this preliminary study was to evaluate the feasibility and the usefulness of EtG determination in VH samples, as a marker of antemortem alcohol ingestion, in a selection of forensic autopsy cases where ethanol determination turned out to be negative in both blood and VH.

METHODS: Postmortem VH and blood samples were collected from subjects fulfilling the following inclusion criteria: no report of putrefaction of the corpse; no evidence of ocular diseases, traumatic lesions, morphological alterations; collection of specimens within 48 h after death; absence of blood in collected VH specimens. Only negative VH and blood samples for ethanol were further analyzed for EtG. Ethanol levels were measured by headspace gas chromatography (GC) equipped with a flame ionisation detector (cut-off 0.1 g/L). EtG levels were determined by liquid chromatography – mass spectrometry (LC-MS) in APCI conditions and using D_5-EtG as internal standard (cut-off 0.10 mg/L).

RESULTS: Twenty-five VH and blood samples were analyzed for EtG. Four blood and corresponding VH samples were both positive for EtG. Three VH samples, whose corresponding blood samples were negative, turned out to be positive for EtG. Positive blood samples (4 out of 25, 16%) showed EtG concentrations ranging from 0.45 to 1.33 mg/L, with a mean value of 1.05 mg/L. In positive VH samples (7 out of 25, 28%) EtG concentrations ranged from 0.15 to 1.50 mg/L, with a mean value of 0.71 mg/L.

CONCLUSIONS: For the first time to the best of our knowledge, this preliminary study showed the feasibility of EtG detection in VH samples. The exclusive presence of EtG in some of the examined VH samples could be interpreted assuming a relatively prolonged EtG half-life in VH, due to its hydrophilicity that prevents permeation across the posterior blood-eye barrier. In spite of further indispensable investigations to elucidate its role, EtG in VH could be considered a marker of antemortem ingestion of alcohol with a longer detection window with respect to blood, and useful to supplement or replace EtG determination in urine, which may be at times diluted or not available postmortem.

Even small amounts of ethanol cause positive ethyl glucuronide results in urine

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AIMS: Testing for ethyl glucuronide (EtG) in workplace drug testing is controversial. The Substance Abuse and Mental Health Services Administration (SAMHSA) state that EtG is too sensitive a biomarker to base legal or disciplinary action on it. Past drinking experiments always dealt with normal to rather high amounts of ethanol. The aim of this study is to measure EtG after the ingestion of small amounts of ethanol.

METHODS: Volunteers consumed either 1 g (n = 7) or 0.5 g (n = 4) of ethanol 1 h before lunch. Urine samples were randomly collected for 12 hours thereafter. Samples were analyzed by two different laboratories. The first laboratory used a method involving sample dilution with the addition of internal standard and analysis on an AB/Sciex QTrap 4000 LC-MS/MS with a LOD and LLOQ of 0.025 and 0.05 mg/L, respectively [1]. The other laboratory used a newly developed SPE procedure using Carboprep200 (Restek) and Cleanscreen EtG carbon (200 mg, USP/Amchro) columns and analysis using a LC-MS/MS system (AB / Sciex API 365) with similar sensitivity [2]. The latter procedure had recoveries that ranged between 60 – 90% (with either column) and a LLOQ of 0.05 mg/L.

RESULTS: For the subjects who consumed 0.5 g ethanol, the results of 10 samples that had EtG concentrations greater than 0.05 mg/L were compared in both laboratories. Variations of single determinations were below 40% for these samples, with two exceptions (with EtG being above LOD in these samples, anyhow). EtG was detected in urine samples for up to 12 h (after 1 g ethanol) and 10 h (after 0.5 g ethanol). Peak EtG concentrations after consuming 1 g and 0.5 g ethanol were 0.35 and 0.15 mg/L, respectively.

CONCLUSIONS: 1) An SPE method for EtG in urine samples has been developed and has been applied in combination with a standard LC-MS/MS system. Similar results could be achieved with this instrumentation as with one of the most sensitive LC-MS/MS systems on the market. 2) Even ingesting as little as 0.5 g ethanol can lead to positive EtG results in urine. In order to avoid false accusations due to use of ethanol-containing products (e.g. mouthwash [3]), appropriate EtG cut-off concentrations for workplace drug testing must be set and donors should be advised about sources of "contamination" and be told to avoid using products such as ethanol-containing mouthwash, "alcohol free" beer and hand sanitizers prior to testing.

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Simultaneous ethyl glucuronide and ethyl sulfate determination in biological fluids using an APCI source operating without corona discharge

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AIMS: Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are non volatile, water-soluble markers of recent alcohol

consumption that can be detected for a lengthy period of time after alcohol has been excreted completely. Most of the analytical procedures concerning EtG and EtS determination are based on liquid chromatography-mass spectrometry (LC-MS) with electro-spray ionization (ESI) sources. As mobile phases with high percentages of water are needed to achieve satisfactory chromatographic separation of EtG and EtS, on axis ESI interfaces may suffer from the heavy burden of high throughput analyses and require frequent maintenance. On the other hand, atmospheric pressure chemical ionization (APCI) sources proved to be less affected by LC mobile phase composition.
The development of a reliable and robust LC-APCI-MS/MS procedure for determining EtG and EtS in blood and urine was considered of interest in a forensic setting where a large number of samples need to be examined. In particular, since the use of an APCI source without corona discharge has recently been proposed, we tested the performance of this approach for the analysis of both compounds.

METHODS: All measurements were taken on an LCQ Duo (Thermo) ion trap under negative ion conditions; multiple MS experiments were performed in multiple reaction monitoring following these transitions: EtG: 221→203/113/75; d_5-EtG: 226→203/113/75; EtS 125→125/98; d_5-EtS: 130→130/98; LC separation was achieved using a Hypercarb 100 x 2.1 mm column by gradient elution of water (1 mM formic acid, 1 mM ammonium formiate) and acetonitrile (3 mM formic acid, 8 mM ammonium formiate) at 0.2 mL/min. Post-column acetonitrile was added at 0.4 mL/min. Calibrators were prepared by spiking blank urine at EtG and EtS concentration from 0.025 mcg/mL to 10 mcg/mL. Real urine samples were collected from University Hospital of Padova in the context of a driving license regranting protocol. Prior to LC-MS analysis, samples (200 mcL) were spiked with internal standards (d_5-EtG and d_5-EtS, 2 mcg/mL) and extracted by solid phase aminopropyl cartridges.

RESULTS: Under classical APCI conditions (i.e. *with* corona discharge) only EtG produced [M-H]- species at m/z 221. Vice-versa, using the same APCI source without any corona discharge, abundant [M-H]- species of both EtG and EtS were obtained. The LC-MS/MS procedure for determining the two analytes in body fluids was consequently developed in APCI *without* corona discharge. Method validation showed, for both analytes, a limit of quantification of 0.05 mcg/mL, intra-day and inter-day coefficients of variation lower than 20% (urine spiked with EtG/EtS at 1 mcg/mL; 5 replicates/day, 2 weeks); no interfering peaks in any blank sample analysed (20 blank urine samples). In addition, the APCI source showed to be much more robust than ESI, with little maintenance needed. Analysis of 100-150 samples/week only demands ion source cleaning, while the LCQ entrance capillary needs to be changed every 5,000 runs or more.

CONCLUSIONS: LC-MS/MS using an APCI source without corona discharge can be considered an effective, reliable and robust analytical method for determining EtG and EtS in blood and urine.

Biological markers of long-term alcohol consumption in alcoholised drivers: gender differences in the determination of carbohydrate-deficient transferrin (CDT) measured by capillary electrophoresis (CDT CE) or direct immunoassay (N Latex CDT) and gamma-glutamyltransferase (GGT)

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AIMS: Alcohol use disorders are one of the most frequent addiction problems and are an important issue in clinical toxicology especially for licence reapplication after driving under the influence of alcohol. Carbohydrate-deficient transferrin (CDT) is a biomarker for chronic alcohol intake and has been reported to be superior to conventional markers like gamma-glutamyltransferase (GGT) and mean corpuscular volume (MCV). Recently, new methods of detection for CDT such as capillary electrophoresis (CDT CE) and direct immunoassay (N Latex CDT) may improve the separation and detection of Tranferrin isoforms. Most research has focused on males and there are few studies on female patients. The main objective of our study was to compare the diagnostic value of CDT and GGT, in male and female alcoholised drivers according to their reported alcohol consumption levels.

METHODS: We studied 490 Swiss drivers referred to the institute of legal medicine because of driving while under the influence of alcohol. These subjects were consecutively recruited in 2003, 2004 and 2006. Alcohol intake was monitored by structured interviews, self-reported drinking habits and the Audit questionnaire as well as information provided by their family and general practitioner. ROC curves were calculated for comparing sensitivity and specificity of the markers. Results were expressed as the mean area under the ROC curve and its 95% CI.

RESULTS: Characteristics of patients: N = 490 (438 men and 52 women). Alcohol consumption: > 2 drinks per day: 134 (27.3%), ≤ 2 drinks per day: 245 (50%), 0 drinks (> 1 month): 111 (22.7%). The comparison between moderate (less or equal to 2 drinks per day) and excessive drinkers (more than 2 drinks) showed that ROC curves area for CDT were between of 0.73 (N latex CDT) and 0.72 (CDT CE) for males and between 0.56 and 0.59 for females. Normal CDT and

GGT levels were defined by the manufacturer as ≤ 2.5% for N latex CDT, ≤ 1.2 for CDT CE and <85 units/l for GGT.

Males

Marker	ROC area	95% CI	Cut-off	Sensitivity*	Specificity*
CDT N latex	0,73	0,67-0,79	2,5%	0,40	0,87
CDT CE asialo + disialo-Tf	0,72	0,66-0,78	1,2%	0,58	0,77
GGT	0,63	0,56-0,69	85 U/l	0,33	0,83

Females

Marker	ROC area	95% CI	Cut-off	Sensitivity*	Specificity*
CDT N latex	0,56	0,38-0,73	2,5%	0,07	0,93
CDT CE asialo + disialo-Tf	0,59	0,41-0,76	1,2%	0,14	0,8
GGT	0,75	0,58-0,92	85 U/l	0,29	0,90

** Sensitivity / Specificity, determined at the cut-off*

CONCLUSIONS: In contrast to GGT, CDT (N latex CDT or CDT CE) was less sensitive in female than male alcoholised drivers for providing effective detection of hazardous alcohol use. This may have important implications for licence reapplication.

The determination of carbohydrate deficient transferrin (CDT) with N latex CDT: comparison with a validated HPLC method

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AIMS: Carbohydrate Deficient Transferrin (CDT) is the collective name of a group of a minor glycoforms of transferrin (Tf) (namely, asialo-, monosialo-, disialo-Tf), whose serum concentration increases after repeated excessive alcohol intake (i.e. 60 - 80 g/day for at least 7 - 10 days). At present, CDT is considered the most reliable indicator of chronic alcohol abuse, showing a diagnostic specificity > 90% and a diagnostic sensitivity >70%. For several years CDT analysis has routinely been performed by immunometric methods based on a preliminary extraction of the CDT isoforms with disposable anion exchange cartridges followed by immunometric detection using antibodies anti-total Tf. This method, however, suffers from poor analytical specificity and scarce precision, as demonstrated by different studies [1]. Quite recently, a new immunometric method (N Latex CDT, Dade Behring, Deerfield, IL, USA) has become available, which, differently from the previous immunoassay, is based on antibodies specific for the CDT related isoforms of Tf. The aim of the present study was to compare the analytical performances of this new analytical approach with a validated HPLC method, at present the candidate reference technique for CDT analysis.

METHODS: Sera from 150 subjects, applying for the driving license after its confiscation for drunk driving, have been analyzed with both techniques. The immunoassay analysis was performed on BN ProSpec(R) system (Dade Behring) using proprietary reagents (N Latex CDT, Dade Behring)). N Latex CDT is a ready to use latex-particle enhanced assay that contains polystyrene particles coated with a monoclonal antibody against CDT that are agglutinated by CDT-coated polystyrene particles. CDT in the sample inhibits the reaction between antibody-coated and CDT-coated particles in a dose dependents manner. The agglutination reaction is monitored by measuring light scattering. HPLC analysis was performed on a gradient HPLC (Shimadzu Europe, Germany), using an anion exchange column (Recipe, Munich, Germany) eluted with a gradient of NaCl (from 0 to 135 mM in 20 minutes) concentration in 10 mM BIS-TRIS buffer, pH 6.2. Detection was by radiation absorbance at 460 nm. Prior to injection, serum samples were saturated with a ferric solution. The results from both methods were expressed as percentage ratio of CDT isoforms on total transferrin (% CDT).

RESULTS AND CONCLUSIONS: With the immunoassay %CDT ranged from 1.03 to 4.5% with mean value of 1.96% (SD 0.63). With HPLC %CDT ranged from 0.8% to 5.6% with mean value of 1.62% (SD 0.92). The difference of the mean values (0.34%) was found statistically significant ($p < 0.001$) using the Student t-test. The quantitative comparison of the results of the two methods showed a significant correlation described by the following equation: $y = 0.5925x+0.997$ ($r = 0.86$) where y = data from the immunoassay and x = data from the HPLC. The analysis of about 100 subjects with normal CDT concentrations, based on the HPLC analysis [2] (cut-off = 1.9%), were used to evaluate a cut-off level of the N Latex CDT assay, which at the 97.5% percentile resulted to be 2.58%.

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Drugs in motor vehicle accidents
Médicaments, stupéfiants et accidents de véhicules à moteur

Characteristics of fatal crashes involving drugs in Victoria and associated contributory factors

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It is well established that alcohol and illicit drugs impair key psychomotor and cognitive functions necessary for safe movement within the road transport system. While the success of random-breath test operations with respect to the apprehension of DWI offenders and reductions in alcohol-related crashes has been well documented, only until recently has it been possible to undertake roadside testing for illicit drugs. It is within this context that this research aimed to determine both the extent of illicit drug use and situations in which drug use is associated with road fatalities in Victoria, Australia, with a view to informing the enforcement process.

In Victoria, the State Coroner is notified of all road deaths, with findings being made after consideration of a range of evidence. Using the National Coroners Information System (NCIS), all drivers, pedestrians, cyclists and motorcyclists aged over 16 with a positive toxicological result were identified, with details extracted and entered into a custom-built database. The NCIS database contains details of coronial files from 1 July 2000, with demographic and contextual data being available, along with the medical cause of death, mechanism of injury, and text reports of circumstances as interpreted by the police, toxicology findings, autopsy findings, and the Coroners finding. Additional crash information was obtained from the VicRoads police-reported casualty crash database, with cases linked by means of probabilistic matching.

For the year 2004, 97 drivers, pedestrians, motorcyclists and cyclists aged 16 and older were identified as having alcohol or a licit or illicit drug present at the time of death. Data from 2004 was used as the closure rate was higher than more recent years at the time the project commenced. Of these 97 cases, drivers of passenger cars represented the highest proportion (56.7%), followed by pedestrians (14.4%), motorcycle riders (11.3%), pick-up truck / van drivers (10.3%), drivers of heavy transport vehicles (5%) and cyclists (2%). Approximately 40% were persons under 30 years of age, 68% were single vehicle crashes, and two-thirds of deaths occurred at the roadside.

Apart from alcohol (49.5% of cases), the most commonly detected drugs were: narcotic analgesics (22.7%); Δ^9-THC (19.5%); anti-depressants (18.5%), anaesthetics (15.4%, 93% hospitalized); benzodiazepines (13.4%), amphetamines (9.2%), heroin metabolites (7%). Only 2 cases were detected with MDA/MDMA and 1 case for cocaine metabolites. High levels of ethanol and THC were detected. Polydrug use was relatively common.

The strength of the NCIS database is that information concerning contributory factors (e.g., vehicle or environment-related factors) to the crash is frequently noted, as well as prior drug use, traffic violations, and crash history. In addition, the narrative information provides rich details of the pre-crash and drug use behaviors, thus enabling targeted and refined enforcement strategies to be developed. Differences in crash characteristics were evident across variables including intersection type and speed zone. These factors will be explored in the written paper and potential implications for enforcement will be discussed.

Driving under the influence of Cannabis. Incidence in fatal road traffic accidents

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Cannabis is one of the most frequently used illicit drugs in the world and its consumption has been maintained more or less homogeneous over time. Consequently, it is frequently found when performing forensic toxicological analyses, including those related to road traffic accidents.

This study has two main objectives: firstly to determine the incidence of cannabis consumption in drivers involved in fatal road traffic accidents, and secondly, to assess if the driver was under the influence of this drug when the accident took place.

A total of 810 fatally injured drivers were included in the study. All blood samples were subjected to a broad toxicological analysis. This includes ethanol analysis by headspace gas chromatography, screening analysis by immunoassay method (CEDIA) and confirmation and quantification by gas chromatography-NPD and gas chromatography-mass spectrometry (GC-MS). Cannabis compounds (THC and THC-COOH) were analyzed by GC-MS with deuterium labeled internal standards. The limit of quantifications were 0.25 ng/mL and 0.50 ng/mL for THC-COOH and THC, respectively. THC-COOH and THC concentrations were evaluated, using Model II from Huestis et al. (1) in order to estimate the time of cannabis exposure.

The results showed that ethanol was the most prevalent substance, since around 50% of the samples tested positive for it. In addition, a high prevalence of cannabis was also noticeable in around 7.5% of the cases. Concentrations ranged from 0.8 to 33.3 ng/mL (mean 8.1 ng/mL) and from 4.4 to 60.5 ng/mL (mean 16.81 ng/mL) for THC and THC-COOH, respectively. By applying Model II calculations, based on THC: THC-

COOH ratio, the estimated time of exposure ranged from 18 to 246 minutes (mean 57 minutes). Looking at the frequency distribution of the time, in the majority of the cases (58%) the time was lower than 60 minutes; while times from 1 to 2 hours and from 2 to 3 hours had an incidence of 28% and 8%, respectively, and in only 3% of the cases was the time higher than 3 hours. If we consider that peak effect is experienced about 15-30 minutes after smoking and that the pharmacological effects often last for 2-4 hours, we can deduce that more than 90% of the killed drivers who had deceased cannabis were under this influence during the accident. From this study it is possible to conclude that cannabis consumption plays an important role in fatal road traffic accidents because there is a high prevalence of this drug among deceased drivers and because they were driving under the influence of cannabis when the accident took place.

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Traffic accident risk associated with the prescription of hypnotic drugs: a registry-based cohort study

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Despite the high prescription rate of hypnotics, there is limited information about traffic accident risk associated with use of these drugs. The aim of this study was to examine whether use of the hypnotics zopiclone, zolpidem, nitrazepam and flunitrazepam was associated with increased traffic accident risk at a national population level. Information on prescriptions, road accidents and emigrations/deaths was obtained from Norwegian population-based registries. All drivers 18 - 69 years old involved in personal injury accidents between January 2004 and September 2006 were identified. Data were linked based on the unique 11-digit identification number assigned to all individuals living in Norway. The hypnotic exposure period was taken to be first week after the day of dispensing the hypnotics. Standardized incidence ratios (SIR) were calculated by comparing the incidence of accidents among subjects prescribed hypnotics with the incidence among unexposed subjects. SIR were also stratified by age and gender. Culpability was not considered. During the study period 135 traffic accidents occurred during zopiclone exposure, 29 during zolpidem exposure, 28 during nitrazepam exposure and 18 during flunitrazepam exposure. SIR for the hypnotics were: 2.4 (2.1 - 2.9) for zopiclone, 3.0 (2.0 - 4.4) for zolpidem, 2.5 (1.4 - 4.1) for nitrazepam, and 4.0 (2.4 - 6.4) for flunitrazepam. The moderately increased SIR for drivers who were prescribed hypnotics was expected on basis of earlier research. It is difficult to conclude whether the increased SIR was related to use of hypnotics, or to the underlying sleep-problem or other confounding factor. For comparison, an analogous study from our group has shown an increased SIR for drivers who had received any prescribed medicines [SIR 1.7 (1.6 - 1.8)].

Cannabis among fatally injured drivers: circumstances surrounding the accident

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Of all illicit drugs, cannabis has always been of particular focus due to its prevalence within the general population, especially among young people. Over the years, research has attempted to shed additional light on the possible threat cannabis poses to road safety. Although prior studies did not establish evidence of an increased risk of crashes, recent studies using more refined methods provide a better understanding of the actual effects cannabis has on driving.

However knowledge on behavioral impairment is still limited [1]. The information contained in the reports of the coroners on circumstances surrounding the accident can lead to interesting conclusions on the type of behavior observed for consumers of cannabis. Besides, the concomitant consumption of cannabis and one or several other drugs (often the alcohol) can bring an escalation of the bad behavior among drivers.

Under the Quebec study on the role of drugs in fatal road accidents, the Coroner's office took blood and urine samples from deceased drivers. Cannabis was detected in 18.5% of the urine samples (n = 541) of deceased drivers. The presence of cannabis was more detected frequently at the young drivers (approximately 50% of the cases were drivers of less than 25 years) and at the men (90%). Accidents were more frequent on weekend days (60%) and equally on night period (9:00 p.m. to 9:00 a.m.) vs day period. Alcohol (> 80 mg%) was also present almost 50% of the cases and we found another drug other than alcohol for 35% of the cases.

Overall results will be presented on the basis of the various components of cannabis (THC and/or metabolites) and the concentrations found. Combined use (cannabis/alcohol and cannabis/other drugs) will be examined along with the circumstances surrounding each accident (type of collision, period, etc.) and the characteristics of the deceased driver (age, sex, responsible/non-responsible).

At the moment, the analysis is in progress. The analysis will be completed by the end of April.

Reference:

Walsh, J.M. Drugs other than Alcohol, research needs and priorities, Transportation research circular no 502, Transportations research board, Jan 2001.

Fatal accident drivers with earlier arrests due to drugged driving

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OBJECTIVE: To investigate the frequency of earlier arrests due to alcohol or other drugs related driving among fatal accident drivers, comparing the group of drivers with and without alcohol or drugs detected in their blood samples collected after the crashes.

METHODS: All investigated fatal accident drivers who died during 2001- 2002 (n = 243) were followed retrospectively back to 1985, to record the frequency of earlier arrests due to alcohol or other drugs related driving. The group of fatal accident drivers was divided in two different groups: 1) Those with alcohol and/or other drugs detected in their blood samples collected after the accident (n = 106), 2) Those with no alcohol/ or drugs detected after the accident (n = 137). The frequency and number of earlier arrests were compared between the two groups of fatal accident drivers.

RESULTS: Group 1) Approximately 30% (n = 32) of the fatal accident drivers who died during 2001-2002 with alcohol or drugs detected in their blood samples, had earlier been recorded for at least one arrests due to alcohol or other drugs related driving. The number of earlier arrests varied from 1 – 22.
For drivers with no alcohol or drugs detected in samples collected after the crashes, less than 3% (n = 4) had earlier been arrested due to alcohol or drugs related driving. The number of earlier arrests varied from 1 – 5. The drugs most frequently detected in samples collected after the accident besides alcohol, were benzodiazepines, tetrahydrocannabinol and amphetamines. Multi-drug detections were frequently found. This pattern of drugs detections was similar to findings in samples collected during the earlier arrests.

CONCLUSIONS: Drivers who die in drugs related crashes have a high probability to have earlier arrests recorded due to alcohol or drug related driving (approximately 1 out of 3), compared with those with no alcohol/drugs detected in samples collected after the accident (approximately 1 of 33). Multi-drug use is frequently found in samples collected after the accident which is similar to drug pattern detected during earlier arrests, indicating drug misuse or abuse. A drug treatment program seems necessary for drivers with frequent arrests and alcohol/drugs detected in their blood samples, in particularly those with more than one drug detected.

Driving behaviour under the influence of Cannabis and cocaine

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INTRODUCTION: The purpose of this study is to describe experiences of driving under the influence (DUI) of cannabis and cocaine among clients in treatment.

METHODS: A questionnaire was administered to clients in treatment for abuse of either cocaine or cannabis, many of whom also had a problem with alcohol; additional groups of clients consisted of those in smoking cessation and gambling programs (N = 1,021). Open-ended and close-ended questions were asked on how cannabis or cocaine affected the clients' driving, collision history, and frequency of DUI of various drugs and combinations.

RESULTS: Two patterns of driving behaviour were found in both qualitative and quantitative analyses for those who drove under the influence of cocaine or cannabis: reduced driving capabilities and more reckless styles of driving. When comparing DUI driving capabilities and reckless style with frequency of DUI of cannabis or cocaine, reduced driving capability from cannabis was inversely related to frequency of DUI of cannabis, but other relationships were not significant. Separate logistic regression analyses, controlling for age, sex, and driving exposure, showed that frequency of DUI of alcohol alone and cocaine or cannabis with alcohol were significantly related to "at fault" collisions; whereas frequency of DUI of cannabis or cocaine alone were not related.

CONCLUSIONS: The findings indicate that both cannabis and cocaine have negative, but different effects on driving. The frequency of DUI of cocaine or cannabis was a risk factor for "at fault" collisions only when these substances were combined with alcohol.

DRUID: driving under the influence of drugs, alcohol and medicines – an integrated research project in Europe

DRUID: conduite sous l'influence de stupéfiants, alcool et médicaments – projet européen de recherche intégré

Driving under the influence of drugs, alcohol and medicines

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The Integrated Project DRUID is assigned to the 6th European Framework Programme and deals with the scourge of impaired-driving. The project aims to gain new insights to the degree of impairment caused by psychoactive substances and their impact on road safety. The objective of DRUID is to give scientific support to the EU transport policy to reach the 2010th road safety target objective of a 50% reduction in the number of road deaths in the EU by establishing guidelines and measures to combat impaired driving. The DRUID consortium consists of 37 scientific institutions from 19 European countries to unite the international expertise of this research area.

DRUID will:

- conduct reference studies of the impact on fitness to drive for alcohol, illicit drugs and medicines and give new insights to the degree of impairment caused by psychoactive substances and their impact on road safety.
- generate recommendations for the definition of analytical and risk thresholds.
- analyse the prevalence of alcohol and other psychoactive substances in accidents and in general driving, set up a comprehensive epidemiological database.
- evaluate "good practice" for detection and training measures for road traffic police allowing a legal monitoring of drivers.
- establish an appropriate classification system of medicines affecting the fitness to drive, give recommendations for its implementation and create a framework to position medicines according to a labelling system.
- evaluate the efficiency of prevention, penalisation and rehabilitation strategies, considering the difficulties of appropriate evaluation schemes for combined substance use and recommend "good practice".
- define driving ban strategies, combining road safety objectives with the need for mobility.
- define the responsibility of health care professionals with respect to patients' consumption of psychoactive substances and its impact on road safety, elaborate guidelines and make information available and applicable for all European countries.

To guarantee a sound and efficient research progress, a comprehensive quality assurance system was implemented within the DRUID project. A joint Quality Assurance Plan will facilitate and assure a unified deployment of internationally acknowledged scientific standards and procedures to provide a thorough empirical basis for European transport policy makers.

A meta-analysis of alcohol studies: an attempt to multi-dimensional risk functions.

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One main objective of DRUID is to establish thresholds for psychoactive substances in traffic. For alcohol, the scientific knowledge about substance concentrations, behavioural effects and driving safety is extremely large. This holds true for experimental as well as for epidemiological studies. Based on this knowledge about behavioural effects and accident risk, the idea is to use alcohol as reference substance for other psychoactive substances like medicines and illegal drugs. For these substances normally quite a lot of experimental results in the lab are available, but only few epidemiological studies with a direct estimation of accident risk. Therefore, an extended meta-analysis for experimental studies with alcohol has been conducted, giving detailed information about the alcohol-induced behavioural impairment. Since for alcohol also a lot of epidemiological studies are available, the linkage between behavioural impairment and accident risk can be determined. Therefore, the ultimate goal is to introduce alcohol as a "gold standard", allowing to estimate the accident risk of a given substance and its concentration by looking at its behavioural effects in controlled experimental studies.

A key problem is to classify the different performance and effect measures used by the experimenters into a conceptual framework, which then allows to link these effects to traffic scenarios. Therefore, the driving task also has to be classified into the same conceptual framework. A new multi-dimensional classification system is established to describe the relevant features of an experimental task (kind of attention, motor complexity, level of processing, etc.), which then can be linked to different aspects of the driving task. A first application will be demonstrated.

For the meta-analysis over 10,000 publications are sighted. From these, about 300 publications are selected by applying certain in- and exclusion criteria. Because each publication contains several findings concerning the effects of alcohol (e.g. effects on different performance dimensions or performance under different blood

alcohol concentrations), the meta-analysis deals with more than 3.000 reported results.

Reference:

This abstract refers to Work Package 1, Methodology, of the project DRUID - Driving under the influence of Drugs, Alcohol and Medicines, duration 15 October 2006-14 October 2010, see www.druid-project.eu

Experimental drug-driving studies in DRUID

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Task 1.2 of the DRUID program is designed to assess the effects of illicit and licit drugs on driving performance under experimental, placebo-controlled conditions. The drugs under study will include medicinal drugs and illicit drugs that have been frequently implied in epidemiological research to potentially increase crash risk; as well as "novel" or "recent" drugs that are suspected to pose a potential hazard to driver safety. The experimental studies constitute a concerted effort to elucidate drug effect on driving performance with respect to 2 major drug categories: i.e. stimulant drugs and hypnotics. The major aims of the studies are:

1) to provide tolerance levels for each individual drug under study;
2) to assess potential drug-alcohol or drug-drug interactions;
3) to assess the effects of drugs on driving as a function of workload ;
4) to cross-validate data obtained from driving simulators and actual driving tests.

Driving performance will be assessed using psychomotor and cognitive tests measuring skills related to driving, driving simulators and on-the-road driving tests. Over-the-road tests will include combined city driving and highways driving test in order to measure performance under varying workload conditions, and will involve the fundamental aspects of driver vehicle interactions (i.e. standard deviation of lateral position, speed, brake reaction time, time to speed adaptation, headway, time to collision). Tests in advanced driving simulators will be developed to assess similar fundamental driving skills, but in addition include tests of reactions on traffic signals, compliance with traffic control devices, risk taking behavior and situation awareness as well. In the current proposal, the effects of stimulants and hypnotics will be assessed both in driving simulators and on-the-road driving tests in order to cross-validate both experimental approaches. For more information on DRUID, see www.druid-project.de

Protocols for road side surveys and hospital studies

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OBJECTIVES: Joint guidelines for road side surveys and hospital studies have been set up for the DRUID project (1). One of the purposes of DRUID is to calculate the prevalence of drink and/or drug driving in the general driving population (13 European countries), the prevalence of alcohol and drugs in drivers who have been seriously injured in a road accident (eight European countries), as well as the relative risk of being injured in a road accident while impaired (the same eight European countries).

METHODS: In road side surveys randomly selected vehicles are stopped by the police. The sample locations and hours must be systematically selected for the resulting samples to be representative of all drivers in a country, if necessary after weighting. The prevalence of drink and drug driving vary between week days and weekend days and between day time and night time. Thus, these periods should be sufficiently covered, with the highest compliance of the police with the planned activities. Drivers of passenger cars and small vans will be asked to deliver a sample. By systematic selection of sites and times and random sampling of vehicles e.g. age and gender of the drivers will be represented by their proportion in the driving population.

The same type of drivers as in the road side surveys will be included in the hospital studies, originating from road accidents on public roads from the same areas as for road side surveys. This will enable us to calculate the relative risk of drink and drug driving. Injured drivers will only be included if the hospital is the primary admission. It is recommended to select the drivers for inclusion if the severity of injuries results in maximal abbreviated injury score (MAIS) 2 or higher, however deviations may occur in some of the countries.

Drink and drug driving will be based on confirmation analysis of samples from all included drivers, either saliva or whole blood in the road side surveys, depending on the possibilities in each country, and whole blood in all hospital studies, collected less than three hours after the accident. The same 23 substances, including alcohol, will be analysed for in both road side surveys and hospital studies.

The following core data will be collected for each driver. For road side surveys: Age, gender, time, date, vehicle type, professional use of car, road type and sample analysis result. Especially in countries where only saliva

is collected, it is recommended to record self-reported drug use and time of use, in order to compensate for negative analyses in case of a positive self-report. For hospital studies: Age, gender, time, date, vehicle type, professional use of car, single or multi vehicle accident, severity of injury (preferably MAIS), medication, fluids, alcohol and drugs taken or administered between accident and sample taking and sample analysis result.

EXPECTED RESULTS: The prevalence of drink and/or drug driving in the general driving population will be calculated in 13 European countries. The prevalence of alcohol and drugs in drivers who have been seriously injured in a road accident will be calculated in eight European countries, as well as the

Reference:

This abstract refers to Work Package 2, Epidemiology of DRUID - Driving under the influence of Drugs, Alcohol and Medicines, duration 15 October 2006-14 October 2010, see www.druid-project.de

Selection of an Oral Fluid Collection Device for EU-project DRUID

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The suitability of different oral fluid (OF) collection devices for EU-project DRUID (Driving Under the Influence of Drugs, Alcohol and Medicines) was tested. The investigated devices were Greiner Bio-One, Orasure Intercept®, Immunalysis Quantisal™, Statsure Saliva Sampler™, Cozart®, Sarstedt Salivette®, Malvern Medical OraCol, Acro Biotech Salicule, Varian OraTube™ and an ordinary plastic tube (Sarstedt). Volume of collected oral fluid, collection time, drug recovery from the devices and stability of the drugs in the collectors in storage were tested.

One ml of OF spiked with different drugs (amphetamine, MDMA, cocaine, Δ9-THC, morphine, codeine, diazepam and alprazolam, all 1000 ng/mL in OF) was added to each device and stored according to the instructions of the manufacturer. The calibration standards and samples were extracted with ethyl acetate at pH 10. The solvent was separated and evaporated. The residue was derivatised with ACN-MSTFA and analysed with GC–MS.

All devices collected over 1 mL of OF, except for Orasure Intercept (mean volume 0.86 mL). As a result, the devices were divided into three classes (Table 1).

The results of the study emphasize the impact of the selection of the OF collection device on the whole toxicological procedure. Considerable differences in the overall reliability of OF collection devices were noted. For the DRUID project, Statsure was selected for the OF collection device. However, the plastic tube was also regarded as an acceptable choice for collection.

Table 1. The results of the study.

Parameter	Best	In Between	Worst
Collection time Best: < 2 min In between 2 - 4 min Worst > 4 min	Quantisal Statsure OraCol Salivette	Oratube Intercept Tube	rest
Recovery Best: > 80% In between: THC < 80% Worst: THC and other(s) < 80%	Statsure	Quantisal Intercept Greiner Salicule Tube	rest
Stability (28 days storage at -20°C) Best: < 15% units decrease In between 15 - 29% units decrease Worst: > 30% units decrease	Cozart (alprazolam failed)	rest	Quantisal Greiner

Toxicological analyses in the DRUID epidemiological studies: analytical methods, target analytes and analytical cut-offs

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Within the epidemiological studies of the DRUID project, 13 laboratories from across Europe will analyse whole blood, oral fluid or urine from the general driving population and injured drivers. In total, approximately 60000 samples will be analysed, making risk assessment possible even for drugs and medicines with low prevalence. However the comparability of results from different laboratories has to be ensured. Therefore collection of samples, analytical methods, target analytes and analytical cut-offs have been standardized for all laboratories involved.

Target analytes were selected based on suspected impairing effects and prevalence. Partners were asked to give their opinion on the relevance of a preselection of analytes. A list of 23 drugs were chosen by at least 9 laboratories and were included in the 'core list' for which analysis is mandatory: ethanol, amphetamine, MDMA, MDA, MDEA, methamphetamine, cocaine, benzoylecgonine, THC, THCCOOH, 6-acetylmorphine, diazepam, flunitrazepam, alprazolam, clonazepam, oxazepam, nordiazepam, zolpidem, zopiclone, lorazepam, morphine, codeine and methadone. In order to include more classes of medicinal drugs, all countries were asked to add at least three more analytes. As a result, 28 additional drugs were included as 'national drugs'. These will be analysed for in 1 up to 8 countries.

It has been agreed upon to collect whole blood in glass Vacutainer-type tubes containing sodium fluoride and potassium oxalate. Oral fluid will be collected using the Statsure™ device. This device was chosen after comparison of 10 collection devices using the following criteria: collected and recovered volume, ease of use for the subjects, collection time, recovery and stability of a selection of the target analytes.

Since only a small volume of sample is available (5-10 mL blood, 1 mL oral fluid), all laboratories have to develop methods for simultaneous detection of the target analytes. All labs agreed to use either LC-MS-MS or GC-MS in SIM-mode.
Analytical cut-offs were established for the core list based on those used in ROSITA-2, SAMHSA cut-off values for oral fluid and recommendations from a NIDA meeting in Talloires.
Because of practical and legal considerations, different samples will be used: whole blood, serum/plasma and oral fluid. Literature on correlation between the analyte concentrations in different body fluids is limited. Different possibilities to establish these functions within DRUID are investigated because this knowledge would increase comparability of results: 1) Between epidemiological (WP2: blood, oral fluid and urine) and experimental studies (WP1: serum and plasma). This traditional difference in study design can be explained by the characteristics of the fluids and the needs of the study. Whole blood is used in the epidemiological studies because legislation in most countries is based on blood and transportation is easier since haemolysis is not a concern (plasma without fluoride preservatives doesn't have haemolysis either but causes cocaine degradation). Sample clean-up of plasma is however easier. 2) Within the epidemiological studies themselves (most countries: oral fluid in road-side survey, blood in hospital study). Solving this problem will however prove to be very difficult, since current data indicate a weak or absent correlation between blood and oral fluid. Therefore semi-quantitative interpretation might be the best possible outcome in some countries.
Proficiency testing for both blood and oral fluid will be organized.

Reference:

This abstract refers to Work Package 2, Epidemiology, of the project DRUID - Driving under the influence of Drugs, Alcohol and Medicines, duration 15 October 2006-14 October 2010, see www.druid-project.eu

Blood spot analysis

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Prescription and illegal drugs may impair driving performance and increase accident risk. However, the relationship with accident likelihood is far from being transparent. The DRUID research project aims to assess the situation in Europe and to calculate the accident risk for drug impaired drivers. In a subset of drivers and in experimental studies, blood samples will be analyzed. Besides conventional sampling in appropriate tubes, dried blood spots will also be prepared.
The use of dried blood spots has been around for more than 40 years. Nowadays, this technique is worldwide applied for the screening of metabolic disorders in newborns and adults, and it is increasingly used in clinical drug analysis. At present, methods that use small amounts of blood applied to filter paper are developed and validated for drugs that may have an effect on the ability of drivers to operate safely in traffic situations. Liquid chromatography/tandem mass spectrometry was used to obtain the metabolic profile including major metabolites or degradation products. The suitability of the dried blood spot technique has been investigated for ester- and amphetamine-type drugs as well as diazepam and major metabolites, comparing results from blood collected on filter paper and in whole blood. It has also been verified that analytes are quite stable in dried blood spots for several days, even under unfavourable conditions (40°C).
The analytical procedures applied were rapid and sensitive with an excellent precision, which is a major concern in the determination of drugs from blood spots. The results indicated superior stability of ester-type drugs over that in a conventional blood sample. Therefore, this sampling technique seems of particular importance if degradation products are not pharmacologically active, if any conclusion is drawn from the pattern of the parent drug and its major degradation products or if degradation products are not easily detected by routinely applied procedures.
The use of dried blood spot assays makes acquisition and transport of samples practical and less expensive. The DRUID project offers the possibility to test the utility of the dried specimen to take a more prominent role in the detection of a drug impaired driver.

Drug-related crash risk calculation in the DRUID project

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A main objective of the EU research project DRUID (Driving Under the Influence of Drugs, alcohol and medicines) is to assess relative risks associated with the use of a number of legal and illegal psychoactive substances by motorists. These risks will be calculated in eight European countries based on a case-control design of the studies. Cases consist of drivers admitted to hospitals' Emergency Departments who have a MAIS (Maximum Abbreviated Injury Scale) score of 2 or higher. The use of this inclusion criterion guarantees a homogeneous group of cases in all participating countries. Controls consist of a representative sample of drivers from the hospitals' catchment areas. In order to obtain a representative control sample from each catchment area, a random sample of drivers will be drawn from moving traffic at a systematic sample of research locations and research times. This kind of epidemiological case-control study was previously used in EU research project IMMORTAL.
Relative risk factors of various psychoactive substances and combinations of substances will be calculated by

computing Odds Ratios. In order to be able to adjust for confounding factors, statistical analysis will be performed by using a logistic regression model, which allows to include covariates like gender, age, road type, season, time of day, and day of the week.
With regard to the case-control study, two major sources of potential bias have to be considered. The first one results from the fact that, in most countries, cases will be blood-tested while controls will be saliva-tested. Drug concentrations found in saliva (or oral fluid), however, cannot be converted into blood concentrations by using a fixed factor. Furthermore, with respect to some frequently used drugs like benzodiazepines and cannabis, saliva analysis is less sensitive than blood analysis. In order to get a better insight into the different outcomes of both methods of analysis, some countries will try to obtain an additional blood sample from drivers who are suspected of being positive for one or more of the drugs under scrutiny. Suspicion can be based on subjects' self-reported drug use, including time of consumption, and/or clinical signs of impairment displayed by them. Another important source of potential bias may be subjects' refusal to be tested. This problem, too, can at least partially be overcome by recording their self-reported drug use and signs of impairment.
The method of the DRUID case-control studies and the associated methodological problems, advantages and disadvantages are discussed, e.g. in comparison with the more classic case-control study method introduced by Borkenstein and still used in the USA, and with case-crossover studies also known as culpability studies.

An attempt to integrate data from different methodological approaches to estimate traffic risk for substances – some theoretical considerations

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DRUID is an integrated project with 36 institutes from 18 European countries doing research on the problem of drugs, alcohol and medicaments in traffic. One of the major objects is to make further proceedings in defining thresholds for drugs and medicaments. Therefore a large number of different studies using different methodological approaches is necessary. Within DRUID the empirical basis to define thresholds is established by three different methodologies.
Epidemiological research is done by several countries in order to obtain information about prevalence rates and risks. Experimental studies are conducted by the partners focusing on the effects of different psychoactive substances on different groups of subjects (including patients) in driving tasks. Different meta-analyses are carried out in order to summarize the effects of drugs, alcohol and medicaments on various aspects of performance in a structured way, which are reported in experimental research literature.
Within the epidemiological approach, roadside studies will provide information about the frequency of driving under the influence, case-control studies will provide information about the risk of having an accident or of being injured under the influence, whereas culpability studies will provide information about the risk of causing an accident. If sufficient information is provided, dose-dependent risks for events of different severity can be calculated (accident, injury, fatality). Experimental studies conducted in driving simulations or at a test track usually do not give information about traffic risks but about the change of the distributions of different driving parameters or errors in different experimental groups. In order to compare experimental results with epidemiological risk information, these distributions of driving parameters have to be transferred to risk measures. One possible procedure will be introduced as an attempt to integrate these both data types. Regarding meta-analytic data some kind of risk estimations can be done by using the vote-counting method. If sufficient publications exist for one substance group significant results of performance impairment can be treated as a probability of a drop-out regarding performance at a certain substance concentration.
Risks originating from the estimations based on these different methodologies will probably not be at the same level, which means that the risk of having an accident in the experimental setting of a driving simulation will be different from the risk of having an accident calculated from case-control studies. Relating e.g. the risk for an alcohol accident based on experimental research to the risk calculated from epidemiological research may lead to appropriate weighting factors to transfer results from one methodological approach to the other. These weighting factors may also be applied for drugs and medicaments to transfer "experimental" risks to "epidemiological" risks Methodological considerations regarding the integration of these different approaches and data types will be presented.

Reference:

This abstract refers to Work Package 1, Methodology, of the project DRUID - Driving under the influence of Drugs, Alcohol and Medicines, duration 15 October 2006-14 October 2010, see www.druid-project.eu

Analytical versus risk thresholds for psychoactive substances – synopsis of the DRUID results

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Within the Integrated Project DRUID different methodologies (for example epidemiological, experimental research) will be applied using a wide variety of observational parameters, for example

experimental variables measuring performance and activation state, accident data based on case control studies or from medical records, experimental on-road data under varying conditions. In order to combine the results from all different studies conducted in DRUID, a theoretical framework and an integration methodology will be established using the results of alcohol research as reference base.
Beside the conduction of experimental research the relevant literature about the effects of those substances on human performance and driving behaviour will be evaluated for their impact on traffic safety. This meta-analysis is weighted for methodological standards of studies and for a quantitative estimation of driving related skill-parameters (for example attention, reaction time, lane keeping). The results of the meta-analysis will be included in the threshold calculation for psychoactive substances together with the outcome of the experimental and epidemiological research.
A total of 15 experimental studies will be designed to assess the effects of drugs, alcohol and medicines on driving performance under experimental, placebo-controlled conditions. Driving performance will be assessed using psychomotor and cognitive tests measuring skills related to driving, driving simulators and on-road driving tests. The studies will include drugs and medicines that have been frequently implied in epidemiological research to potentially increase crash risk; as well as ”novel” or ”recent” drugs which are supposed to affect driving performance but where not enough knowledge exists. The studies will be conducted with the psychoactive substances alone and in combination. To preserve as much as possible the driver’s mobility under medical treatment the experimental studies will compare the driver fitness of ill persons which have to take their medicines with and without these medical treatments.
Based on the results of epidemiological research, odds ratios regarding the accident risk for various drugs and medicines can be computed for different substance concentrations and thus contribute to setting concentration thresholds.
Taking into account the consumption-driving patterns of substance users, the prevalence of substances among road users in general traffic and in accident causation, the experimental studies results and the results of relative risk calculation from epidemiological studies will be integrated in the established theoretical framework. Recommendations for thresholds for the substances under investigation as a proper indicator of impaired driving comparable to the promille thresholds for alcohol (alcohol per se limits) will be formulated according to the results.
The last step in this work package will be a synopsis of the different results and the knowledge which was collected and summarised in this IP concerning the recommendation for further regulations. This synopsis also implies a comparison of the results with respect to the different legal conditions in the European Member States. This includes the prevalence of substances as well as the adequacy of recommended thresholds.
Reference: This abstract refers to the project DRUID – Driving under the influence of Drugs, Alcohol and Medicines, Sixth Framework Programme, contract No TREN-05-FP6TR-S07.61320-518404-DRUID, see http://www.druid-project.eu.

The DRUID project and the categorization of medicinal drugs and driving

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A large proportion of the population habitually drives while taking medicinal drugs. The prescription of medicinal drugs is an everyday factor in clinical practice, and even though safer and more effective medicinal drugs are being commercialized every day, some of them can deteriorate psychomotor performance, which can affect a person’s ability to drive safely.
The DRUID Work Package Classification (WP4) has four objectives:

- To review the existing classification/categorisation systems and labelling systems regarding medicinal drugs and driving.
- To propose and agree on the criteria and the methodology for the establishment of a European classification/categorisation system and labelling system of medicinal drugs and driving.
- To develop a methodology to continuously update the classification/categorisation system and labelling system of medicinal drugs and driving.
- To propose a classification/categorisation system for the relevant therapeutic groups of medicines available in the market.

For the achievement of these objectives, DRUID Work Package 4 has issued four research tasks. Task 4.1: Review of existing classification efforts; Task 4.2: The establishment of criteria for a European categorisation, based on expert consensus; Task 4.3: Establishment of a framework for the classification/categorisation and labelling of medicinal drugs and driving; Task 4.4: Coordination and synthesis report.
Four deliverables are expected as a result, including a review of the existing classification/labelling systems (Deliverable D.4.1.1, available in month 15, start of DRUID project, October 15th, 2006), the proposal of a methodology to achieve the criteria for a European categorisation (Deliverable D.4.2.1, month 39), to provide a classification/categorisation system for the relevant therapeutic groups of medicines available in the market, including newly available drugs during the timeframe of the project DRUID (Deliverable D.4.3.1, month 45), and the classification of medicinal drugs and driving: a synthesis report (Deliverable D.4.4.1, month 46).

The partners involved in the DRUID Work Package 4 Classification are: University of Gent (Belgium, task leader of Task 4.1), University of Groningen, Pharmacy (The Netherlands, task leader of Task 4.2), Bast (Germany), University of Grenoble, Centro Regional de Pharmacovigilance (France), Centre for Research and Technology Hellas (Greece), and the University of Valladolid (Spain, task leader of Task 4.3) acting coordinator of DRUID WP 4.
The work package classification will have an output both for physicians/pharmacists and other health professionals, as well as the patients taking these medicinal drugs, through two major actions: categorization of the medicinal drugs on driving ability, and the proposal of appropriate labelling systems regarding medicinal drugs and driving. This abstract refers to the Work Package 2, Classification, of the project DRUID – Driving under the influence of Drugs, Alcohol and Medicines, Sixth Framework Programme, contract No TREN-05-FP6TR-S07.61320-518404-DRUID, duration 15 October 2006-14 October 2010, see http://www.druid-project.eu.

Legal strategies for secondary prevention of DUI and DUID: the role of license withdrawal and conditions for reinstatement

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OBJECTIVES: The European Council Directive of 29 July 1991 on Driving Licenses calls upon all Member States to provide regulations that prevent dangers in traffic safety resulting from alcohol- and drug-impaired drivers. Implementation of this directive into the different national laws implicated that the legal systems regarding these issues are only partly comparable but show substantial differences, evoking secondary problems (e.g. "License Tourism"). Thus the Workpackage 6 "Withdrawal" of the EU-Project DRUID aims to collect information regarding legal issues and practices of license withdrawal and reinstatement in the European countries. The different issues concerning criminal sanctions and administrative consequences will be assessed and compared, intending to evaluate the effects of the various strategies. An exploratory focus will lay on conditional withdrawal and driving licenses with restrictions, e.g. while under medication treatment or concerning technical systems like ignition interlocks. These analyses serve to gain insight into methods to reduce impaired driving while preserving the greatest mobility and on the same time reduce the accident risk while impaired. The integration of all findings and the results of WP5 "Rehabilitation" and WP1, Task 1.3 "Recommendation of thresholds" will lead to the development of prototypical solutions and comprehensive recommendations for a catalog of legal countermeasures against DUI/DUID in Europe and will provide reliable data for the orientation of administrators, politicians and researchers.

METHODS: The study adapts from the questionnaires which were sent out in 1998 and 2001 by the Pompidou Group to 24 European countries. The results are going to be updated by sharpening the questionnaires with respect to withdrawal and reinstatement issues. Additional specifications regarding technical measures for secondary prevention will be included. The redesigned version will be sent to legislative administrators of all Member States and selected non-member states in order to collect and evaluate data on best practices. Interviews with country experts serve to complete all information. Workshops will be arranged and the evaluated results will be presented and discussed by representatives and experts of different countries.

RESULTS: The activities of this WP will start in 2008. The presentation will give an overview over the scope of the problem and will compare the main issues of interests (e.g. administrative vs. criminal law) to similar research fields, e.g. legislation on alcohol interlocks in North America. Further it appeals to all international experts to cooperate and thus support the activities of the DRUID-Project's WP6 "Withdrawal".

CONCLUSIONS: The outcomes will serve as draft recommendations for future legislation regarding license withdrawal and reinstatement in the European Union.

Dissemination, guidelines and professional standards

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The objectives of this work package (WP 7) in DRUID are to review the state-of-the-art and documented effectiveness of existing campaigns and practice guidelines regarding psychoactive substances focussed on the general public and health care professionals, the development of information materials aimed at the general public and health care professionals and a proposal for improving the procedures for assessing fitness to drive within the framework of Council Directive 91/439/EEC (on driving licences).
Several methods are used for investigating the impact of existing campaigns such as searches of the scientific literature and consultation of experts through various international organisations.
Various documents and brochures for dissemination of information will be addressed to the general public, drivers as patients with a focus on younger drivers,

physicians and pharmacists, policy makers and other public bodies.

The existing medical guidelines for assessing fitness to drive will be evaluated on the basis of legal outcomes in the event of accidents occurring after a positive decision from a physician's side. After reviewing some best practices a proposal for implementing improvements in legislation and procedures will be presented.

The implementation of new practice guidelines and protocols for medical and pharmaceutical care derived after input form other Work Packages of DRUID, will be investigated after baseline measurements in the Netherlands, Belgium, Spain and Germany. Specific attention will be given to opportunities of using Information and Communication Technology (ICT) in the computerised information systems that physicians and pharmacists use in their daily practice.

The outcomes of all tasks in this Work Package will offer opportunities to evaluate the effectiveness of risk communication to patients and drug consumers regarding psychoactive substances affecting driving performance. By using the categorisation system developed in WP 4 and by investigating the patients' and health care providers' satisfaction the outcomes of the implementation can be further defined.

The European project DRUID - Scientific support for combating impaired driving

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The objective of DRUID (Driving Under Influence of Drugs, alcohol and medicines) is to give scientific support to the EU transport policy to reach the road safety target of reducing the number of killed road users in the EU by 50% between 2001 and 2010 by establishing guidelines and measures to combat impaired driving. DRUID consists of seven Work Packages:

WP0 "Management" embraces a number of governance tasks: overall co-ordination and scientific management; financial management; quality assurance; liaison between the Commission, the Steering Committee and partners; development and implementation of Consortium Agreement; representation of the Consortium; management of intellectual property rights; organisation of dissemination activities; reporting, etc.

WP1 "Methodology and research": The aim of WP1 is to establish a theoretical framework and an integrative methodology. Different experimental studies with psychoactive substances will be conducted to assess driving performance and estimate the risk for different substances and to formulate recommendations for thresholds.

WP2 "Epidemiology": The objectives of this work package is to assess the situation in Europe regarding the prevalence of alcohol and other psychoactive substances in drivers in the general traffic and drivers involved in injury accidents, to calculate the accident risk for drug impaired drivers and to identify characteristics of drug impaired drivers.

WP3 "Enforcement": The objective of WP3 is to evaluate roadside testing devices both from a scientific perspective and an operational police perspective. A cost-benefit analysis will be carried out to find out which on-site screening device will have the best cost-benefit rate and to what extend enforcement of driving under the influence of psychoactive substances is cost beneficial for society.

WP4 "Classification": This WP reviews the existing classification and labelling systems regarding medical drugs and driving and a proposal will be made for the criteria and the methodology of a European system. Furthermore, a methodology will be developed to update this system continuously.

WP5 "Rehabilitation": The overall aim of work package 5 is to increase knowledge regarding rehabilitation of drivers with drunk-driving or drug-driving offences. The research will provide fundamentals for establishing adequate and effective rehabilitation measures throughout Member States according to uniform defined criteria and quality standards.

WP6 "Withdrawal": The objectives of this work package are to review the state-of-the-art , collect and evaluate practices in various European countries based on the former related studies, assessment of the effect of various strategies regarding withdrawal of driving licence with focus on the conditional driving license withdrawal and development of recommendations with a comprehensive view on the entire problem.

WP7 "Guidelines and dissemination": The objectives of this work package are to review the state-of-the-art and documented effectiveness of existing campaigns and practice guidelines regarding psychoactive substances, focussed on the general public and health care professionals, development of information materials for general public and health care professionals and developing proposals for improving procedures for assessing fitness to drive.

Workplace drug testing and alternative matrices
Dépistage en milieu professionnel et matrices non-conventionnelles

The effects of storage conditions on the clinical parameters of specimen validity testing in urine collected for workplace testing

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AIMS: The Mandatory Guidelines for U.S. Federal Workplace Drug Testing Programs provide specimen validity testing criteria to identify urine specimens that have been submitted by donors attempting to suborn the drugs of abuse testing process. Specimen validity testing criteria have been established for the invalid, adulterated, substituted, and diluted categories. The invalid categories that utilize clinical characteristics of urine for criteria cut-offs include pH ≥ 3 and < 4.5, pH ≥ 9 and < 11, and creatinine < 2 mg/dL with specific gravity > 1.0010 but < 1.0200 or creatinine ≥ 2 mg/dL with specific gravity ≤ 1.0010 or ≥ 1.0200. Thus, invalid specimen criteria are particularly important since the factors causing such invalid specimens can affect drug stability and hence drug detection.

Since the Mandatory Guidelines became effective in November 2004, a number of specimens with results within the upper pH invalid limits, typically in the range of 9.1 to 9.3, have been reported with no known evidence of donor tampering. The objective of this study was to determine if these increased urine pH findings were the result of exposure to increased environmental temperatures during specimen standing and transport.

METHODS: A freshly collected, random (untimed) urine specimen pool was divided into aliquots that were kept unpreserved at various storage temperatures (-20, 4, 25, 37, and 93oC) for a maximum of two weeks. On each specified day (days 1, 2, 3, 7, 8, 9, 10, and 14 post-void), the pH of each aliquot was measured in duplicate using a pH meter. In addition, the urine stored for two weeks at each of the storage temperatures was analyzed for urine creatinine and specific gravity.

RESULTS: Increasing storage temperatures were found to be associated with the increasing urine pH, with the magnitude of the change related to both storage time and temperature. The pH values of specimens stored at -20°C were relatively constant. Urine stored at 25°C or higher achieved pH results > 9. None of the storage conditions investigated produced a urine specimen with a pH > 9.5. Degradation of nitrogenous urine analytes, such as creatinine, urea, and uric acid, is most likely responsible for the noted in vitro increases in pH.

After two weeks of storage, urine specific gravity was stable at all the tested storage temperatures. Conversely, urine creatinine was stable at –20, 4, and 25°C and unstable at increased temperatures.

CONCLUSIONS: Specimen validity testing criteria are important to identify those urine specimens which will have decreased drugs of abuse recoveries because of pH changes, either caused by environmental storage conditions or attempted adulteration, that invalidate urine testing.

Stability of analyte's precursor(s) and of internal standard must be considered in method validation experiments

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AIMS: Stability of analytes during sample storage/preparation, as well as on autosampler, is a critical issue for correct interpretation of results. However, too often analysts disregard to recognize that analyte's stability involves also the potential degradation of one or more of its precursors present in the sample. In addition, stability of internal standard (IS) should also be considered. The determination of heroin metabolites in biosamples is an excellent example of how these issues may affect quantification. 6-acetylmorphine (6AM) - not to speak of heroin - may be prone to chemical hydrolysis during sample processing. In addition, 3- and 6-morphine glucuronide may be hydrolysed by pH variations. Such phenomena obviously affect not only quantification of these metabolites but also of their degradation product morphine (M). The situation is even more complicated if the respective isotope labelled ISs are added to the sample, as they follow the same degradation pattern as the unlabelled compounds (e.g. D_3-6AM γ D_3-M).

METHODS: A mathematical model was set up in order to assess how and to what extent a degradation precursor γ product during sample processing may affect quantification of both compounds and what precautions should be taken in order to minimise such effects. The model takes into account different kinetic orders (0, I or II order) and rates of degradation, different relative initial concentrations, and the use of isotope labelled ISs or not.

RESULTS: By simulating a quantification experiment of 6AM and M with the corresponding deuterated ISs, 5 calibration points (analytes together) plus blank, 3 controls and 10 samples at different relative concentrations of precursor/analyte, with a total run time of 0.5 h, and assuming that 1000 ng/mL of 6AM are reduced to 900 ng/mL within 24 h during sample processing (including storage in the autosampler) following a I order kinetic and that no degradation of M occurs, accuracy deviation can be as high as -2,3% for M, whereas in the case of 6AM is obviously 0%. Accuracy deviation can be as high as -20,8% and 4,3% for 6AM and -6,8% and -3.4% for M simulating a 0 or II order kinetic of hydrolysis, respectively. Depending on the kinetic type, on the target analyte (precursor/product) as well as on the expected relative concentrations in the sample, the adoption of separate calibration curves for precursor and product or the use of a stable, unlabelled IS may reduce accuracy deviation. However, in the latter case accuracy deviation due to the chemical-physical differences between analyte and IS should be also considered.

CONCLUSIONS: Stability experiments for method validation should be carried out on real positive samples as well as on spiked samples where the analyte and, if known, its precursor(s) aren't put together. In addition, the kinetics of degradation during sample processing of the different compounds involved must be studied in order to adopt the quantification strategy less prone to error.

Development and production of hair reference materials for use as control and calibration for hair drug testing

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AIMS: The Center for Forensic Sciences (CFS) at RTI International is now producing hair reference materials under an NIJ Award, 2006-DN-BX-K012, with objectives to improve the resolution and sensitivity of forensic analytical tools, as well as to enhance the productivity and portability of methods used in forensic laboratories.

METHODS: CFS surveyed laboratories performing hair testing to determine which controlled substances are of most value to forensic laboratories and developed appropriate reference materials accordingly. Four reference materials at predetermined target concentrations are being produced in 2007. Some target concentrations are applicable to current screening and confirmatory testing, while others are for confirmatory testing only.

Head hair strands (14-20 g) were purchased, determined to be drug-free for analytes of interest, and washed to remove potential surface contaminants using an extended phosphate buffer wash. Darker hair samples that were not chemically treated and determined to be in good physical condition were utilized. Each reference material used hair from one individual. Fortification solutions were prepared with appropriate analytes and the intact hair strands were completely submerged in the solution at room temperature for a period of time that was concentration and analyte dependent. Aliquots were removed periodically during fortification process to test for analyte concentration. At the completion of the fortification process, hair was again decontaminated with an extended phosphate buffer wash. The hair was divided into 100 mg aliquots and placed in glass vials for storage. The four reference materials and the theoretical target concentrations are as follows:

Reference material 1	Reference material 2	Reference material 3	Reference material 4
Morphine (500 pg/mg)	THCA (0,3 pg/mg)	Cocaïne (1500 pg/mg)	Amphetamine 750 pg/mg)
			Methamphetamine (750 pg/mg)
			MDMA (750 pg/mg)

Analyte concentrations in these materials will be verified through internal and external testing utilizing multiple forensic laboratories that routinely perform hair testing. Vials submitted to reference laboratories were chosen using a stratified random sampling scheme across the aliquoting process. These laboratories use standard testing procedures including extraction of drug analytes from hair matrix and analysis by GC-MS, GC-GC/MS, GC-MS/MS or LC-MS/MS.

RESULTS: Preliminary results from the hair testing laboratories indicate that Reference Materials 1 (morphine) and 2 (THCA) are within 20% of the theoretical target while Reference Materials 3 (cocaine) and 4 (amphetamines) are substantially higher than the theoretical target. Reference testing and establishment of the uncertainty measurement are on-going and will be reported during TIAFT.

CONCLUSIONS: The implementation of matrix-matched reference materials for hair at appropriate concentrations will further substantiate quality control measures of laboratories and improve the defensibility of their analytical results.

Abuse of zolpidem in racing cyclists: a new type of addiction demonstrated by hair analysis

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AIMS: During several trials in France in the past years, it has been claimed that cyclists can abuse zolpidem

(Stilnox), an hypnotic, for sedation during periods of time off. To document the abuse of benzodiazepines and hypnotics, particularly zolpidem, we have analyzed hair collected from cyclists from the same team.

METHODS: Hair was collected in one day from 29 cyclists during a medical survey and stored at ambient temperature until analysis. The laboratory was requested to test for anabolic steroids, drugs of abuse, corticoids, β-adrenergic compounds (e.g. salbutamol, clenbuterol) and, if sufficient specimen is available, for benzodiazepines and hypnotics. Enough material remained for 12 cyclists. The method included decontamination of hair with methylene chloride, cutting the hair into small pieces followed by incubation of 20 mg in phosphate buffer (pH 8.4). Liquid-liquid extraction, with 1 ng diazepam-d5 as the internal standard, was performed with diethyl ether/methylene chloride (80/20). Separation was performed by LC using a XTerra C18 column with detection by MS/MS. The limits of quantification for all benzodiazepines and hypnotics ranged from 0.5 to 5 pg/mg using a 20-mg hair sample.[1]

RESULTS: From the 12 cyclists tested, 10 were positive for zolpidem (0.3 to 1918 pg/mg), 6 for bromazepam (3.6 to 58 pg/mg), 5 for zopiclone (5.3 to 142 pg/mg), 3 for tetrazepam (7.0 to 139 pg/mg), 2 for diazepam (1.0 and 1.9 pg/mg) and finally 1 for 7-aminoflunitrazepam (79 pg/mg). This clearly demonstrates multi-drug use. Only one single cyclist was found negative. No doping agent was detected during the general investigations.

It is well known that many athletes experience some form of stress that may result in insomnia during the night before the competition. According to cyclists, as regards to the performance capacity, there is no risk to use sleep inducers the night before a race. The ”toxicology of victory” has promoted new behaviors, where performance is the key point, even after the competition, during social life, for example. As athletes are sometimes subject to having their biological clock in disarray, they can develop over-consumption and dependence to active molecules.

CONCLUSIONS: Many cases of drug addiction in athletes have been revealed in recent years. The stress of competitive sports often leads to a specific vulnerability of sportsmen to addiction. In cyclists, zolpidem was the most frequently drug detected with a broad spectrum of exposure, ranging from one-time use (low pg/mg) to long-term use (> 1 ng/mg).

Reference:

Villain et al – Screening method for benzodiazepines and hypnotics in hair at pg/mg level by LC-MS/MS. Journal of Chromatography B, 825: 72-78 (2005)

Hair analysis using LC-TOF MS – A simple and sensitive method for the detection of medical drugs and drugs of abuse

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AIMS: Hair analysis is well established as a tool for the diagnosis of illegal drug abuse. However, the screening for medical drugs e.g. to confirm patients compliance or to detect forensically relevant CNS depressants is not a routine procedure in most laboratories. The present study was performed to evaluate whether simple methanol extraction in combination with liquid chromatography – electrospray – time-of-flight mass spectrometry (LC-TOF MS) would be sufficiently sensitive to detect psychiatric drugs in hair after therapeutic dosages.

METHODS: Hair samples were collected from 35 psychiatric patients receiving 26 different medical drugs. Extraction of 50 mg hair was performed after washing and cutting by overnight incubation in methanol. The evaporated and reconstituted extracts were analyzed using Agilent LC TOF MS. This is a modification to our routine procedure for the analysis of basic drugs of abuse where propionylation increases the chromatographical performance and sensitivity of certain analytes (e.g. morphine) and where an automatic peak finding algorithm in combination with library assisted identification and quantification is used.

RESULTS: Methanol extraction is fast, simple, and effective and LC-TOF MS requires no sophisticated method setup yielding a simple and robust procedure. Validation showed limits of detection (LOD) for 16 drugs of abuse and 35 medical drugs in the range of 0.005 to 0.1 ng/mg hair (above 0.02 ng/mg only for 6 substances). Lower limits of quantification were 0.02 ng/mg or less for 30 substances. From 133 prescriptions of therapeutic drugs, 111 (83.5%) could be confirmed in the hair extracts by accurate mass and retention time. Surprisingly, 151 additional psychiatric drugs were detected and 29 drugs of abuse (in 11 patients) indicating previous use or additional medication. The prescribed drugs not detected were low-dose or taken only for a short period of time (e.g. Lorazepam, Olanzapine, Biperiden) indicating insufficient sensitivity for occasional ingestion. However, in 6 of 7 cases where only a documented single dose of drug was administered the drug could be detected in hair (Haloperidol (n = 3), Chlorprothixene (n = 1), Doxylamine (n = 1) and Flunitrazepam (n = 1)).

CONCLUSIONS: The simple combination of methanol hair extraction with LC-TOF MS analysis provided

sufficient selectivity and sensitivity to detect therapeutic dosages of 28 medical drugs e.g. for confirmation of therapy compliance. However, though LODs were quite low single administrations e.g. in cases of drug assisted assaults would most probably not be detected.

Hair – The novel specimen for routine coroner's toxicology

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IMS: Throughout 2004-2006 hair was taken in addition to the usual specimens of femoral vein blood, urine, and gastric contents in 286 coroners' cases submitted for analysis. The aim of the study was to review the data from hair analysis and from conventional samples in these cases with a view to identify the case types where hair analysis, either alone or in combination with the results from other specimens, assisted the pathologist in establishing the cause of death and/or the coroner in reaching a verdict.

METHODS: The Toxicology Unit at Imperial College London has published a method using GC-MS in SIM mode for the simultaneous quantification of opiates, amphetamines, the cocaine group and diazepam/ desmethyldiazepam from one 20-50 mg sample of hair [1]. By injecting a further aliquot on to the GC-MS using full scan, the same extract can also be screened for unknowns. To date 24 drugs have been identified using full scan including among others two anticonvulsants (carbamazepine, phenytoin), eight antidepressants (amitriptyline, citalopram, dothiepin, fluoxetine, mirtazapine, paroxetine, sertraline, venlafaxine) and three antipsychotics (olanzapine, quetiepine, thioridazine).

RESULTS: Analysis of hair has been found to be useful in identifying the following scenarios:

1) Compliance with prescribed medication. A 22 year old woman was seen to walk on to the train track, the train struck her and she died from multiple injuries. The deceased was being treated for depression and was prescribed citalopram. A 12 cm length of hair submitted for analysis was divided into 4 equal segments. Citalopram and its metabolite were detected in each of the segments.

2) For verifying long term drug use or demonstrating absence of it. A 29 year old male was found dead in the bathroom; a used syringe was found underneath the body. The deceased was a former addict, but the family believed he had not used heroin in the last three years. The results of analysis of postmortem blood demonstrated ingestion of a potentially fatal dose of heroin in combination with ethanol, dihydrocodeine, and cocaine, but analysis of a 5 cm length of pubic hair was positive only for dihydrocodeine and cocaine plus its metabolites.

3) Demonstrating presence or absence of tolerance. An 18 year old male was found dead in bed. The postmortem blood morphine concentration was 0.32 ug/mL, morphine and 6-MAM were detected in urine, but morphine only at a concentration of less than 0.4 ng/ mg was detected in hair.

4) Demonstrating the widespread use of cocaine and its role in: a) Depressive episodes and suicide. Death occurred as a result of self-suspension in 36 of the 286 cases. Analysis of hair showed the presence of cocaine plus metabolites in 15 of these 36 cases, but in eight of the 15 cases no cocaine or metabolites were detected in the postmortem blood or urine; b) Sudden unexplained death relating to heart abnormalities. Five cases were identified where death was due to heart abnormalities with the analysis of hair demonstrating chronic use of cocaine; c) Cocaine-associated excited delirium. Three cases were identified in which hair analysis showed chronic cocaine and the postmortem blood concentrations were within the range associated with recreational use; d) The commission of crime. Among the 286 cases were eight victims of homicidal stabbing and two of homicidal shooting. Analysis of hair showed that five of the eight stabbing victims and both shooting victims had a history of chronic cocaine use. Postmortem blood was submitted with five cases and all were negative for cocaine.

CONCLUSIONS: Analysis of hair provides a reliable and helpful drug history for the pathologist and coroner. This is supporting evidence for establishing the cause of death and the verdict in a whole range of coroners' cases. Analysis of hair provides evidence to indicate deaths due to chronic drug use.

Reference:

Cordero R, Paterson S. Simultaneous quantification of opiates, amphetamines, cocaine and metabolites and diazepam and metabolite in a single hair sample using GC-MS. J Chromatog B. DOI: hhtp://dx.doi.org/10.1016/j.jchromb.2006.12.021

Results of a pilot oral fluids performance testing program in the United States

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AIMS: Since 2000, the Center for Forensic Sciences at RTI International has been conducting a Pilot Oral Fluids (OF) Performance Testing (PT) Program for drug testing laboratories. This program was funded through the Substance Abuse and Mental Health Services Administration's National Laboratory Certification Program (NLCP) contract, with the objective of developing appropriate OF samples and assessing the capabilities of participating laboratories

to perform confirmatory testing for drugs of abuse in OF. Examination of data from this program in 2006 led to a redesigned approach that focused specifically on within- and between-laboratory variability. This study presents the quantitative performance of laboratories participating over a period of nine months in the 2006 OF PT program.

METHODS: A total of 16 laboratories participated in the study. Of these, 14 laboratories participated for the entire study period. OF samples were manufactured in a synthetic OF matrix, and sent to the laboratories as neat, frozen 10 mL aliquots. Samples were formulated such that potentially cross-reacting drug analogs were not contained in the same sample. Three sets of samples were manufactured, with two sample types in each set. Set 1 samples contained methamphetamine/codeineandamphetamine/morphine; set 2 samples contained cocaine/methylenedio–xyamphetamine (MDA) and benzoylecgonine/ methylenedioxyethylamphetamine (MDEA); and set 3 samples contained tetrahydrocannabinol (THC)/ phencyclidine (PCP) and 6-acetylmorphine (6AM)/ methylenedioxymethamphetamine (MDMA). The three sample sets were sent to the laboratory in three cycles, one every three months over the nine-month period. For each cycle, the laboratories were instructed to dilute the samples using the same dilution factor as their specific OF collection device, screen the samples once, and confirm the samples five times under five separate calibrations.

RESULTS AND CONCLUSIONS: Results demonstrated low within- and between-laboratory variability over the course of the study. For some drugs, a significant improvement was observed in analytical variability. For all drugs, the group means were very close to the targeted concentrations. Results indicated that the material was stable over the nine-month period. The table presents the group means and the group %CVs for each cycle.

Drug	Target ng/mL	Mean Cycle 1 ng/mL	% CV Cycle 1	Mean Cycle 2 ng/mL	% CV Cycle 2	Mean Cycle 3 ng/mL	% CV Cycle 3
AMP	75	78,7	13 %	76,8	13 %	75,6	8 %
MOR	60	60,9	20 %	56,0	8 %	57,6	8 %
COD	60	59,1	9 %	58,1	7 %	58,1	9 %
MAMP	75	78,0	13 %	76,4	10 %	75,2	8 %
COC	30	30,0	10 %	29,4	9 %	31,1	12 %
BE	30	28,7	14 %	29,1	12 %	28,8	14 %
MDA	75	77,8	12 %	76,6	10 %	77,1	10 %
MDEA	75	77,6	12 %	78,0	8 %	79,8	7 %
THC	6	6,7	22 %	6,0	23 %	6,0	18 %
6AM	6	5,8	10 %	5,6	11 %	5,6	11 %
PCP	15	15,0	10 %	14,7	13 %	14,7	17 %
MDMA	75	74,4	18 %	75,2	9 %	77,4	12 %

Analytical evaluation of a new oral fluid sample drugs of abuse diagnostic system

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AIMS: For on-site testing, e.g. roadside testing, the desire to perform a drug test has been hampered by the inability to collect an adequate test specimen. As a potential alternative to urine screening, oral fluids can be tested to reveal the presence of pharmacologically active drugs in an individual at the time of testing. Significant correlation has been found between oral fluid concentrations of drugs of abuse and behavioral and physiological effects. Results indicated that oral fluid screening can provide valuable diagnostic information in various situations, including testing at the roadside. This publication describes the development of the new Dräger DrugTest® 5000 System.

METHODS: The point of collection testing system (POCT) comprises a rapid on-site immunoassay (IA), intended for use with an opto-electronic analyzer for the qualitative detection of substance abuse such as cocaine metabolites, opiates, amphetamines, methamphetamine, benzodiazepines and, specifically,Δ^9THC in oral fluid samples. The test-kit combines a sampling system, immunochemical assays and a test-cassette as a "multitask- item", minimizing the user interaction and increasing the overall system performance. Parameters as sampling time and sample amount were evaluated by collecting 117 individual samples from patients in drug treatment centers. The POCT-assay sensitivity, specificity an accuracy were defined by analysis and evaluating up to 503 individual patients oral fluid specimens collected with the new device. The confirmation was performed by laboratory GCMS-analysis of parallel oral fluid samples from the same individuals; *Benzodiazepines verified by commercial ELISA test.

RESULTS AND CONCLUSIONS: This 1st evaluation showed a median sampling time of 64 sec and a median sample volume of 318 mg Oral Fluid (CV: 16%). The following table summarizes the analytical performance compared to the GCMS-data:

Individuals screened	POCT - IA	Cutoff [ng/mL]	Sensitivity [%]	Specificity [%]	Accuracy [%]
503	COC	20	86	99	98
441	OPI	40	90	98	97
341	Δ9THC	25	76	99	93
155	AMP	50	n.a.	99	99
155	METAMP	25	n.a.	99	99
194	*BENZO	15	74	98	97

The results achieved and exceed target values e.g. for collection precision, sampling time, assay sensitivity, specificity and accuracy, as set by state-of-the-art oral fluid DOA screening devices. The 74% sensitivity for BENZO is directly related to the broad spectrum of this drug consumed in the screened population; the cross-reactivity of the evaluated POCT-test and the commercial ELISA varied. There is no prevalence for AMP and METAMP in the screened population.

Comparison of an ELISA microplate assay with LC-MS/MS for the detection of benzoylecgonine in oral fluid

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AIMS: The aim of the work reported here was to assess and compare the performance of the Cozart Oral Fluid ELISA cocaine metabolite assay used semi-quantitatively with a sensitive LC-MS/MS method for the analysis of cocaine related compounds with the testing of oral fluid collected from a criminal justice study.

METHODS: Oral fluid samples were collected from 429 arrestees using a Cozart oral fluid collection system, which collects 1 mL of oral fluid followed by dilution in 2 mL of buffer. The samples were analysed by ELISA on an automated Dynex DSX system. The screening of 25 µL of oral fluid was conducted semi-quantitatively by using a multi-point calibration curve for benzoylecgonine (0, 15, 30 and 150 ng/mL). For confirmation analysis, 200 µl of sample was extracted by solid phase extraction (SPE) using a mixed mode SPE cartridge. Analysis of cocaine, benzoylecgonine, ecgonine methyl ester (EME) and cocaethylene was performed using a Varian LC-MS/MS. Each analyte was determined by multi reaction monitoring (MRM) of two transitions per ion (cocaine m/z 304 to 182 and 105; benzoylecgonine m/z 290 to 168 and 105; EME m/z 200 to 182 and 82, cocaethylene m/z 318 to 196 and 150). Deuterated internal standards were used for the quantitation of each analyte. Calibration standards at 0, 5, 15, 30, 90, 180 and 360 ng/mL were used, and each sample, standard and control was spiked with deuterated internal standard at 120 ng/mL. The LOQ of the method was 3 ng/mL, with a coefficient of variation of 7%.

RESULTS: Of the 429 specimens analysed by LC-MS-MS 48% were negative, 52% were positive for benzoylecgonine, 34% were positive for cocaine, 14% were positive for EME and 1% were positive for cocaethylene. A comparison of the semi-quantitative screening result with the benzoylecgonine concentration determined by LC-MS/MS gave a very good correlation with an r2 value of 0.86. With a screening cut-off of 15 ng/mL and an LC-MS/MS cut-off of 5 ng/mL the performance of the microplate was also assessed and the sensitivity was 96.4%, the selectivity was 98.4% and the accuracy was 97.3%.

CONCLUSIONS: The Cozart ELISA cocaine metabolite assay used semi-quantitatively compared favourably with LC-MS/MS results for benzoylecgonine.

A superparamagnetic particles-based lateral flow immunoassay for benzodiazepines in oral fluid

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AIMS: The need for rapid, sensitive and robust point-of-contact testing for drugs of abuse in oral fluid is becoming increasingly important and we are presenting here a preliminary investigation using a novel approach to meet these always more demanding requirements.

Superparamagnetic particles have been proposed as an alternative reporter system to colloidal gold and latex particles that are commonly used in immunochromatographic tests. These particles are magnetic only in a magnetic field and reading devices measure the total signal generated by the analyte rather than the signal generated from the surface as is the case with optical sensing devices. Benzodiazepines are frequently given in low doses for therapy –typically 2 to 10 mg per day which can lead to very low levels in oral fluid. We report here the development of a magnetic particle-based immunoassay for the detection of benzodiazepines at very low levels.

METHODS: Superparamagnetic particles (300 nm) with amino or carboxyl functional groups were coated with monoclonal anti-benzodiazepines antibodies by covalent binding. The conjugated particles were diluted in detergent containing buffer and incorporated into a standard nitrocellulose-based immunochromatographic lateral flow test strip having bovine serum albumin-benzodiazepine conjugate as the antigen. Binding of the anti-benzodiazepine antibody-magnetic particles conjugates to BSA-BZO in the presence of drug-negative and drug-positive oral fluid was detected using Magna Biosciences Magnetic Assay Reader (MAR). Neat saliva from 10 drug-free donors was spiked with 0.03, 0.3, 1 and 10 ng/mL Temazepam and diluted in buffer. The results were compared with those obtained using colloidal gold particles-labelled anti-BZO antibodies coupled with optical sensing system (Cozart RapiScan Reader).

RESULTS: Anti-BZO antibodies were successfully linked to the superparamagnetic nanoparticles without loss of antibody activity or particle aggregation. The conjugates were tested and optimised with respect to the dilutions and the running conditions of the test. The tests were initiated using 75 µL of diluted oral fluid (1:3) and the development time was 5 mins. Using oral fluid samples spiked with varying concentration of temazepam, 0.5 ng/mL of the drug was detectable

by the magnetic reader system, which is a significant improvement on the 30 ng/mL seen with the current RapiScan test.

CONCLUSIONS: The superparamagnetic particles-based assay described in this work shows a marked increase in sensitivity when compared with the more usual gold and latex particles-based assays. The main advantages that this system would provide include the ability to detect lower concentrations and increase the window of detection, which is essential for analytes like benzodiazepines, absence of interferences and reduction in the sample volume required for performing the test. The sensitivity now achieved is close to the cut-off levels of confirmation methods such as LC-MS (2 ng/mL) or LC-MS/MS (0.2 ng/mL). Further investigation is required on drug-positive donors.

An LC-MS/MS method for the determination of 13 antidepressants and metabolites with preliminary data comparing plasma and oral fluid concentrations

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AIMS: The aim of this study was to develop an LC-MS/MS method for the simultaneous analysis of amitriptyline, imipramine, clomipramine, fluoxetine, paroxetine, fluvoxamine, sertraline, citalopram and venlafaxine and some of their metabolites (nortriptyline, desipramine, norclomipramine and norfluoxetine). This methodology is being used to assess the relationship of the antidepressant concentrations found in plasma and oral fluid.

METHODS: The sample (200 µL of plasma or oral fluid conditioned with sodium acetate buffer pH 3.6) was extracted with an automated solid-phase extraction system, using mixed mode OASIS MCX cartridges. Chromatographic separation was performed using a reverse phase Sunfire C18 IS column (20 x 2.1 mm, 3.5 µm). The mobile phase consisted of acetonitrile and 2 mM ammonium formate used in a gradient mode. Under these conditions, all of the compounds eluted in less than 5 minutes with a total run time of 8 minutes.
To assess the degree of correlation of antidepressant concentrations between both types of specimens, plasma and oral fluid samples were collected from patients on antidepressant treatment in two different weeks. Oral fluid samples were collected by direct spitting.

RESULTS: The method was fully validated, including linearity (2-4 to 500-1000 ng/mL), within-day and between-day precision (CV < 15%), accuracy (MRE < 15%), limit of detection (0.5 ng/mL) limit of quantitation (2 - 10 ng/mL), recovery, relative ions intensity, matrix effect and stability after 3 freeze/thaw cycles (CV < 20%, except for sertraline (CV = -33.4% in oral fluid at the higest studied concentration)). The patients whose samples were proccessed were in treatment with venlafaxine (n = 6), citalopram (n = 6), paroxetine (n = 4), sertraline (n = 3), fluoxetine (n = 3), amitriptyline (n = 2) and clomipramine (n = 1). In this preliminary study, samples collected on only two different occasions were analyzed to evaluate which compounds may provide a good correlation. Correlation between plasma and oral fluid concentrations were calculated by linear regression for each compound inter and intraindividually. Data for the different compounds indicate that there is not a good correlation for any of the them when the results were analyzed interindividually. The best result was for fluoxetine (r^2 = 0.739) and the worst for citalopram (r^2 = 0.0114). Neither was any correlation found for any of the compounds when data were analysed intraindividually, except for venlafaxine (r^2 = > 0.90) in five out of the six patients).

CONCLUSIONS: A fast method was developed and fully validated for the analysis of the most commonly marketed antidepressants in plasma and oral fluid. To our knowledge, this is the first LC-MS/MS method that allows the analysis of these compounds in oral fluid samples. A research project to evaluate the relationship of antidepressant concentrations between these two matrices has been started. Preliminary data when analysing plasma and oral fluid samples intraindividually in two consecutive weeks indicate a possible good correlation between venlafaxine levels in both matrices. Our next step will be to extend the number of samples from patients on venlafaxine treatment to confirm or rule out a good correlation between plasma and oral fluid.

Drug and metabolite concentrations in a large oral fluid database in the UK

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AIMS: Increased testing of oral fluid specimens for drugs of abuse has resulted in generation of extensive databases, but data from these sources have rarely been reported. We collated concentration data from a commercial database created in the UK composed of 8,679 confirmed positive results. The goal was to characterize drug concentrations found in oral fluid specimens.

METHODS: The confirmed positives originated from 635,000 specimens collected from May 2004 to October 2006. Specimens were collected with the Intercept® oral fluid collection device, screened by enzyme immunoassay, and confirmed by GC-MS or GC-MS/MS.

RESULTS: Concentration data (oral fluid in buffer; 1:3 dilution) are listed in the table.

Drug Group	Analyte	N	Mean (SEM), ng/mL	Median (ng/mL)
Amphetamines	Amphetamine	455	2451.3 (156.6)	1162.1
	MDMA	29	241.0 (54.8)	111.7
	MDA	11	77.1 (18.2)	49.0
	Methamphetamine	6	34.8 (10.8)	28.6
Benzodiazepines	Nordiazepam	774	5.9 (0.4)	2.9
	Diazepam	673	111.4 (28.4)	2.8
	Oxazepam	225	2.5 (0.2)	1.4
	Temazepam	117	130.9 (108.8)	2.9
	Chlordiazepoxide	29	24.3 (13.6)	2.4
	Lorazepam	8	120.1 (113.8)	4.7
Buprenorphine	Buprenorphine	263	433.3 (82.6)	24.3
	Norbuprenorphine	173	5.0 (0.7)	2.0
Cannabinoids	THC	714	6.6 (0.7)	2.3
	Cannabidiol	299	5.0 (0.7)	1.6
	Cannabinol	261	2.8 (0.5)	0.9
	THCCOOH	78	0.07 (0.01)	0.05
	11-HO-THC	41	0.35 (0.3)	0.04
Cocaine	Benzoylecgonine	1193	97.1 (5.4)	32.8
	Cocaine	1175	223.2 (24.8)	17.2
	AEME	150	210.1 (72.6)	31.0
	Cocaethylene	80	22.7 (3.9)	12.4
Methadone	Methadone	998	432.2 (36.9)	193.7
	EDDP	300	62.0 (8.7)	14.6
Opiates	Morphine	4575	178.9 (4.4)	49.8
	Codeine	3820	210.5 (103.6)	27.6
	6-Acetymorphine	3554	383.2 (23.9)	31.3
	6-Acetylcodeine	1431	123.9 (12.2)	18.3
	Heroin	1091	580.7 (140.5)	41.6
	Dihydrocodeine	925	1306.2 (381.6)	164.0

CONCLUSIONS: We conclude that a rich array of information on the prevalence and disposition of drugs in oral fluid is revealed by analysis of oral fluid databases.

Screening and quantification of the twenty-four drugs in oral fluid relevant for road traffic safety by means of liquid chromatography tandem mass spectrometry with electrospray ionization

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The non-invasiveness of sample collection is crucial in epidemiological studies of drivers when testing for the presence of abused and medicinal drugs in their bodies. The quantification of drugs is of latter importance due to correlation with blood concentration being not well established in many cases and contamination of the oral cavity potentially leading to incorrect conclusions. Simultaneous screening and quantification of key drugs in oral fluid is a concern in many toxicological laboratories. The aim of this research was to develop a method for the detection and quantification of the following drugs: morphine, 6-MAM, codeine, tramadol, methadone, amphetamine, metamphetamine, MDA, MDMA, MDEA, cocaine, benzoylecgonine, alprazolam, diazepam, nordiazepam, flunitrazepam, clonazepam, lorazepam, oxazepam, carbamazepine, imipramine, zolpidem, zopiclone, and THC.

Solid phase extraction of 1 mL oral fluid using Oasis HLB (30 mg) column, with slight modification of the original manufacturer's procedure was applied. To the eluate collected in deactivated conical glass vial 25 µL of 1% (v/v) HCl in methanol was added before evaporation step. Dry residue was reconstituted in 100 µL of mobile phase consisted of 0.1% (v/v) formic acid in acetonitrile (A) and water (B). Chromatographic separation was accomplished on a LiChrospher RP-select B column (125 x 2 mm I.D.) using gradient elution. The gradient started from 35% A and 65% B and ended on 80% A and 20% B. The flow rate of 0.25 mL/min was switched to 0.5 mL/min in the final segment of the gradient program allowing both the elution of the last drug (THC) in 12.65 min and faster column equilibration. Ions created in electrospray (ESI) chamber were monitored in MS segmental program based on selected reaction monitoring (SRM) and selected ion recording (SIR). For quantification deuterated analogues of all drugs were used.

Method specificity was established through the analysis of 10 oral fluid samples obtained from non-drug taking individuals. The limits of detection (LOD) based on lowest level calibrator and mean noise were from 1 ng/mL to 5 ng/mL. The limits of quantification (LOQ) were appropriate to Talloires and DRUID recommendations, e.g. between 1 and 25 ng/mL. The method was linear to overdose concentrations of all compounds. Medium level recoveries ranged from 36% (morphine) to 99% (oxazepam) for all studied analytes. Poor fragmentation, especially for benzodiazepines, led to employment of SIR mode, which appeared to be more sensitive for these drugs. Pseudomolecular ions and isotopic ions (M+3) were recorded.

Developed method was simple and fast. Special attention had to be paid to reference saliva preparation. Frozen and thaw cycle followed by centrifugation was applied before standard spiking. This was especially important for preparing THC saliva controls. Approximately 50% of added THC concentrations were measured in untreated saliva samples. The probable reason for this is THC precipitation and adsorption on container walls. The method was used for testing saliva samples taken from randomly stopped drivers as a part of European Integrated Project (Contract No TREN-05-FP6TR-S07.61320-548404-DRUID).

Fingernail ICP-MS multi-elementary determination - a useful biomarker of metal or metalloid exposure

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AIMS: Metal and metalloid determination in blood and urine is the most common application of biological monitoring for screening and diagnosis of these elements exposure. Like hair, nails have many superficial advantages as a biomarker for metal or metalloid exposure. Their collection is non-invasive and simple and specimens are very stable, not requiring special storage conditions. The sample size necessary for analysis is small. Nail metal or metalloid content is considered to reflect long-term exposure because this compartment remains isolated from other metabolic activities. Moreover nails are less affected by exogenous contamination than hair.

METHODS: A thirty-two metal and metalloid inductively coupled plasma-mass spectrometry (ICP-MS) procedure using a Thermo Elemental X7CCT series is validated in nails for: Li, Be, B, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Ba, W, Pt, Hg, Tl, Pb, Bi, U. After warm water and acetone decontamination, 20 mg of nails were digested at 20°C with pure nitric acid and diluted (nitric acid, butanol, triton) before analysis. In and Rh were internal standards. Normal values were assessed in 120 volunteer fingernails.

RESULTS: Linearity was excellent and correlation coefficient was higher than 0.99 for all elements. Detection limits ranged from 0.00004 µg/g (U) to 0.5 µg/g (B). The intra-assay and inter-assay inaccuracies, measured as the variation coefficient were below 5 and 10% respectively. The 120 fingernails median values ranged from 0.0002 µg/g (Pt) to 105 µg/g (Zn). All elements show log-normal distribution. Lacking Certified Reference Materials in nails, an adequate quality assessment scheme was ensured by a unique interlaboratory exercise (Institut National de Santé Publique du Quebec, Sainte Foy, Canada), the results of which showed good consistency for elements tested. Six exposure cases to various elements are presented (Pb, Hg).

CONCLUSIONS: Fingernail ICP-MS multi-elementary determination is a very useful metal or metalloid biomarker with various clinical and forensic applications: occupational, environmental, domestic or criminal exposure to these elements.

Alcool testing technology
Technologie du dépistage de l'imprégnation alcoolique

What interfering substances are there in breath of apprehended drivers? experience using a 5-filter infrared analyzer (the Evidenzer)

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Most of the latest generation of instruments approved for evidential breath-alcohol testing incorporate infrared spectroscopy as the analytical principle. The integrity of the results of such testing is sometimes questioned and the claim is made that volatiles other than ethanol in the breath led to an artificially high reading. This defence argument relates to the specificity of the infrared analyzer and its response to interfering substances.

Human breath is composed of oxygen, nitrogen, carbon dioxide and is saturated with water vapour and includes trace amounts of volatile organic substances (VOCs), mainly ethanol, methanol, acetone, carbon monoxide, methane and isoprene. These VOCs are either produced naturally in the body (endogenous volatiles) or inhaled with the ambient air breathed or alternatively might be ingested with food, drink or medication taken.

The concentrations of VOCs in breath are normally vanishingly small and thus have no practical relevance for challenging the reliability of breath-alcohol tests. However, under some circumstances, such as in certain metabolic disorders (e.g diabetes) or after drinking denatured alcohol preparations the concentrations of acetone and isopropanol can increase appreciably.

The Swedish police perform between one and two million roadside breath alcohol screening tests annually. All positive results (> 0.02 g% or 0.02 g/210 L) are followed by an evidential breath-alcohol test using the Evidenzer, which incorporates four infrared (IR) filters at wavelengths between 3.3 - 3.5 micron, and one reference filter at 3.8 micron. The four measuring filters respond to C-H stretching frequencies in any VOCs in breath, including ethanol. During the calibration procedure the signals from the four filters are adjusted to give the same response when ethanol and saturated water vapour are in the breath sample. Accordingly, if other volatiles are encountered during human subject testing and these absorb radiation within the 3.3 - 3.5 micron range, the measurement system most likely is no longer balanced. The instrument reacts by subtracting from the ethanol concentration an amount which is proportional to the degree of interference. If the amount subtracted exceeds a certain threshold value

the instrument will abort the test and report "too much interfering substance". Whenever this happens, the police request a specimen of venous blood for forensic laboratory analysis.
Venous blood is analyzed by headspace gas chromato–graphy and acetone, acetaldehyde, isopropanol, methanol and methyl ethyl ketone (MEK) if present are easily identified. Acetone, isopropanol and MEK were the volatile substances most frequently observed in blood when the Evidenzer had indicated an interfering substance. In most instances the blood and breath samples also contained ethanol above the legal limit for driving. Acetone arises from ketogenesis in those suffering form diabetes or after a prolonged fast or eating low carbohydrate diets for losing weight. Consumption of denatured alcohol spiked with isopropanol also results in elevated concentrations of acetone in blood and breath. Both acetone and MEK are additives to certain technical alcohols sold in Sweden (e.g. T-Red), which is sometimes consumed by drunk drivers.
The argument that interfering substances are common during evidential breath-alcohol testing was not supported by this investigation.

Breath alcohol testing, quality assurance for today's legal environment: Alabama's approach

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For many decades, evidence generated by breath alcohol testing programs has played an influential role in Driving Under the Influence (DUI) cases throughout our nation. Today's legal environment, both in and out of the courtroom, continues to evolve in complexity and sophistication. A byproduct of this evolution is the increased awareness of universal quality control and quality assurance practices. This increased awareness is made evident by the demands and expectations placed on breath alcohol testing programs by the nation's courts. To answer these challenges, the Alabama Department of Forensic Sciences Breath Alcohol Testing Program, utilizing the Draeger Alcotest 7110 MK-III, incorporates multiple layers of quality control and quality assurance practices.
Each individual instrument used in this program is subjected to continual scrutiny to ensure strict compliance with all quality control and quality assurance performance standards. Technological advances in software and hardware are largely responsible for the increased ability to monitor an instrument's performance. The incorporation of Time of Test quality control testing and hardware monitoring coupled with complete data retention has been shown to bolster the credibility of the breath alcohol test result. Additionally, a comprehensive review of each individual breath alcohol test is completed each week. This quality assurance procedure allows for the identification of data trends and enhanced ability to identify possible sources of error. Those would include the environment, the instrument operator, the testing subject, or the breath alcohol instrument itself. Lastly, each instrument is subjected to a comprehensive annual evaluation which demonstrates the instrument's ability to produce scientifically defensible results when used in the field.
The result of implementing forensically sound quality control and quality assurance practices has been a marked decrease in the time spent in court. An overview of this program's multi-layer quality control and quality assurance practices will be presented.

A novel approach to detecting mouth alcohol

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While alcohol in the mouth exists whenever there is alcohol in the blood, the term "Mouth Alcohol" which is commonly referred to with regard to breath alcohol concentration analysis refers to a condition where the concentration of alcohol in the mouth and/or upper respiratory tract is higher than the alcohol concentration in the end expired breath. Mouth Alcohol, if present in high enough concentrations, can falsely bias the accurate measurement of end-expiratory breath alcohol.
For purposes of practical testing for breath alcohol concentrations in DUI enforcement, it is important to either utilize a testing procedure that reduces the likelihood of the Mouth Alcohol effect, or identify when Mouth Alcohol is present so that testing can be aborted for the period of time while the condition exists.
Traditionally utilized Mouth Alcohol detection algorithms identify mouth alcohol by determining if a higher alcohol concentration existed at the beginning of the expired breath sample than at end of the expiration. A breath sample where no Mouth Alcohol exists will produce an ever increasing ethanol concentration, albeit the increase is at a decreasing rate over time.
The traditional mouth alcohol waveform is influenced by a number of factors.
1.The dead space volume of the instrument.
2.The flow rate of the sample.
3.The volume of the sample.
4.The difference in concentration between the alcohol concentration in the mouth versus what is in the end expiratory breath.
This talk will present data that demonstrates how long upper respiratory alcohol concentrations remain higher than end expiratory alcohol concentrations.This talk will discuss a novel approach to determining if Mouth Alcohol exists in expiratory breath using CO2 concentrations from the breath as a reference against which the alcohol concentration can be compared. Since the nature of the O2/CO2 transfer between the blood and deep lung breath is a similar process to the EtOH

transfer from the blood to breath, it stands to reason that the CO2 concentration and EtOH concentrations should both be at their peak concentration in the alveolar breath. The approach which will be discussed assumes that if the EtOH concentration reaches a plateau or peak prior to the CO2 concentration reaching a peak or plateau, there is an indication that mouth alcohol exists. Data from tests demonstrating the technique, a discussion about its advantage over existing techniques and a review of the techniques current limitations will all be addressed in this discussion.

Use of pocket-model breath alcohol testers by DUI offenders: a survey of purchase and utilization issues

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OBJECTIVE: Because alcohol consumers' ability to subjectively self-assess blood alcohol concentration (BAC) has been found to be notably poor (Silverstein, Nathan & Taylor, 1974), they sometimes make irresponsible decisions, such as driving a motor vehicle while impaired by alcohol (Jones & Lacey, 2001). Pocket-model breath testers (PMBTs), a relatively new category of personal breath test devices, offer drinkers the opportunity to obtain an objective assessment of BAC (Reed, 2006; Stellin, 2001). Smaller than preliminary breath testers (PBTs) used by law enforcement, these battery-powered devices provide users with numerical readout of BAC and range in cost from $40 to $120 USD.

Although the accuracy and precision of PMBTs have been evaluated (Van Tassel, 2004), there is a lack of research into the actual utilization of these devices by consumers of alcohol. The goal of this exploratory study is to investigate purchase and utilization issues associated with alcohol offenders' use of PMBTs.

METHODS: Two groups of participants were surveyed regarding numerical readout PMBTs' acceptable pricing, purchase likelihood, intended frequency of use, overall utility, and impact on consumption. The DUI group (n = 32) consisted of drivers recently convicted of driving under the influence of alcohol (DUI). The comparison group (n = 72) consisted of drivers not convicted of DUI.

RESULTS: Compared to the comparison group, participants in the DUI group were 43% more likely to purchase a PMBT, and were willing to pay 21% more to acquire a device. The DUI group participants indicated that they would utilize PMBTs more often than the comparison group participants, and rated the overall utility of the devices 16% higher. Additionally, DUI group participants were 51% more likely than comparison group participants to state that PMBTs would lead users to consume less alcohol than they would without the devices.

CONCLUSIONS: The DUI group participants' higher ratings on purchase likelihood and acceptable product pricing suggests that they are receptive to tools such as PMBTs to prevent recidivism and avoid the monetary and other costs associated with future alcohol offenses. That DUI group participants would expect to utilize PMBTs more often than comparison group participants suggests that drivers convicted of DUI might be more likely to employ the devices during each drinking episode. Implications for future research include actual purchase and use rates, and how PMBTs are utilized under actual drinking conditions to prevent alcohol-impaired driving.

Forensic breath alcohol calibration laboratory accreditation – a program administrator pro and con analysis

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Historically, breath alcohol testing used in traffic safety in the United States was often seen as an enforcement tool with its oversight given to police personnel rather than a forensic analysis under the supervision of scientists in the laboratory. Forensic breath alcohol testing is arguably the most litigated of all the forensic disciplines and as legal defenses continue to grow in complexity, many in the forensic community, police command structures and the judiciary have begun to recognize the need for establishing breath alcohol testing as an area of forensic specialty. Along with the development of this forensic specialty comes the need to establish scientific standards.

Forensic crime laboratories have routinely embraced laboratory accreditation as a means of demonstrating they meet established standards for management, personnel, operational/technical procedures, equipment and physical facilities. The American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB), which currently accredits forensic disciplines including controlled substances, toxicology, trace evidence, forensic biology/DNA, firearms/ toolmarks, questioned documents, latent prints, crime scene, and digital evidence, is in the process of developing an accreditation program for breath alcohol calibration laboratories using the calibration laboratory standards as per the ISO17025 program.

This presentation will provide an overview of breath alcohol program administration in a laboratory / legal environment and explore the pros and cons of such programs seeking accreditation of their calibration laboratory facilities. The concept of accreditation will be explored and comparisons made to existing quality assurance approaches.

Detection of ethanol in exhaled breath condensate: a preliminary study

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Ethanol is a central nervous system depressant and widely consumed drug. Although it has been extensively studied in gaseous exhaled breath (referred to as breath), little is known regarding the disposition of ethanol in exhaled breath condensate (EBC). EBC is collected by condensing exhaled breath vapor onto the walls of a chilled collection device, such as the commercially-available Jaeger ECoScreen. This project studies the measurement of ethanol in breath from a unique perspective, one that requires multiple breaths for a single EBC specimen. The results would highlight the importance of breathing patterns in understanding the relationship between blood and breath ethanol concentrations.

In this preliminary study, 4 human participants (3 males, 1 female) were enrolled on separate days. Prior to the consumption of an ethanol-containing beverage, all subjects provided a baseline set of specimens, which included breath, EBC, blood and oral fluid. Breath ethanol concentrations were determined in real-time by the Intoximeters AlcoSensor IV device. All other specimens were collected and stored for ethanol analysis by an automated headspace gas chromatography with flame ionization detection technique. The EBC was collected with a user-modified ECoScreen device that isolated deep lung gas and condensed it at a temperature near 10°C. Venous blood was collected using a phlebotomy technique with an IV catheter placed into the left arm or hand of each individual, and oral fluid was collected using Sarstedt Salivettes.

The ethanol dose administered to each subject was calculated based on the subject's weight, and the subject was not to exceed a blood ethanol concentration of 0.10 g/dL. The beverage was consumed within 40 minutes, and the subjects had minimal food in their stomachs. Breath ethanol concentrations were monitored for the remainder of the study using the AlcoSensor IV device. During the post-absorptive phase, multiple sets of specimens were taken in a similar manner as the baseline specimen set.

Ethanol was detected in EBC of subjects after consuming the ethanol-containing beverage. The inter-individual EBC-to-blood ethanol ratio ranged from 1.5 - 3.9. This ratio is highly dependent on the subject's breathing patterns including respiratory rate and volume, and its variability can be minimized by employing standardized breathing patterns.

Although there is inter-individual variability, results of this preliminary suggest a potential intra-individual correlation between alcohol concentrations in EBC and venous blood when normal breathing patterns are sustained. Correlations between concentrations in blood, oral fluid and breath during the post-absorptive state are consistent with published values.

An analysis of 'source code' litigation in the United States: what challenges have been asserted, and where is this litigation heading?

W.C. HEAD, T.E. WORKMAN

Challenges to the inner workings of forensic breath analyzers have emerged in numerous states. These challenges center around having computer software experts analyze the computer "source code" (the programmed operating sequence commands that control the software code) of the state-approved devices in the respective jurisdictions. The exact legal grounds for the litigation have vary, but most raise issues of criminal discovery, confrontation of the State's evidence and due process of law. A plethora of legal obstacles by the breath device manufacturers have been raised. These generally challenge the necessity of allowing such inspection, or seek to impose "trade secret" claims or patent confidentiality issues as a means of preventing the turnover of the source code materials. This presentation will review the legitimacy of some of these challenges and the status of current litigation and will seek to predict how these challenges will impact law enforcement confidence levels for forensic breath testing equipment that is currently being used in DUI-DWI cases across America.

The four major breath instrument manufacturers for United States forensic testing are CMI (Intoxilyzer® brand devices); National Patent (BAC Datamaster® brand devices), Intoximeters, Inc. (Intoximeter® brand devices), and Draeger Safety, Inc. (Draeger Alcotest® brand devices). The presentation will discuss specifics of litigation in the primary states that court cases are pending (New Jersey, Arizona, Florida, Georgia and others) and will compare and contrast the widely varying stance (i.e., from an "open records" approach by National Patent, to all-out warfare by CMI) being taken by the different major instrument manufacturers. In addition to identifying the history and progression of current litigation, this presentation will identify key technical concepts and issues relating to computer source code "average defect density" and identify the various parts of computerized forensic breath testing devices and the relationship these parts have to the other critical parts of these devices. Also, the presentation will explain why concepts of constitutional due process and confrontation of evidence will eventually compel review of the various instruments' source codes. One premise of this presentation is that judicial review of

reliability of these devices is critical to both public confidence in the integrity of these forensic tests as well as assuring that per se alcohol convictions are supported by verifiably accurate scientific analysis under a variety of testing conditions.

Transdermal alcohol monitoring

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CONTEXT: Transdermal alcohol monitoring is a technique designed to monitor alcohol consumption by measuring the alcohol content of insensible perspiration excreted through the skin over a given period of time. This technique was the basis for the development of a new alcohol monitoring bracelet containing a fuel cell. More recently, alcohol monitoring bracelets have become an increasingly popular way to monitor impaired driving offenders, however many criminal justice professionals implicated in the use of these devices have limited knowledge of the science supporting this technique.

OBJECTIVE: This paper provides a summary of the scientific literature on transdermal alcohol and transdermal alcohol monitoring devices and describes what is known about continuous transdermal alcohol monitoring, what is not known, and future research needs.

METHOD: Review of the scientific literature.

RESULTS: The transdermal excretion of alcohol has been studied and understood since 1936. The principles that apply to breath testing also apply to transdermal testing. After 70 years of peer-reviewed research, it has been clearly established that ingested alcohol can be validly measured in perspiration. Transdermal testing can qualitatively discriminate between consumption of none, small, moderate and large amounts of alcohol. Transdermal alcohol measurements are not intended to provide precise, quantitative estimates of alcohol consumption similar to evidential tests. Moreover, transdermal testing involves a measurable delay in absorption and elimination of alcohol. As such, simultaneous blood or breath testing and transdermal testing should not be expected to produce a similar BAC reading.

CONCLUSIONS: To date, there have been few evaluations of Secure Continuous Remote Alcohol Monitors (SCRAM), the only commercially available alcohol monitoring technology at this time. While, these initial evaluations demonstrate that officers and offenders involved in these studies generally approve of the technology and believe it has merit, more large-scale quantitative surveys and case-control studies are needed to corroborate findings and answer questions regarding impact on offenders.

Transdermal alcohol detection in the laboratory and in the field

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This report is an evaluation study of two types of transdermal devices that detect alcohol at the skin surface representing two types of electrochemical sensing technology. The Alcohol Monitoring Systems (AMS) SCRAM™ ankle device and the Giner WrisTAS™ wrist device were worn concurrently for the evaluation by 22 paid research subjects (15 males, 7 females), for a combined total of 96 weeks. Each subject participated in both laboratory drinking to .08 grams per deciliter (g/dL) BAC and normal drinking on their own. A total of 271 drinking episodes with BAC ≥ .02 g/dL were logged: 60 from laboratory dosing, and 211 from self-dosed drinking. Both devices detected alcohol at the skin surface as expected but each has its own unique weak points and strong points. The SCRAM unit has security features and automated reporting protocols that make it suitable for the offender market, whereas the WrisTAS unit is a research prototype that has had trials as an aid to detection in alcohol treatment settings. Neither unit had false-positive problems of note. False negatives were defined as TAC (transdermal alcohol concentration) response < .02 g/dL when true BAC ≥. 02g/dL. Overall, the true-positive hit rate detected by WrisTAS was just 24 percent largely due to erratic output and/or the device not retaining recorded data. This outcome occurred during 67 percent of all drinking episodes and likely reflects a faulty chipset rather than a faulty sensor. SCRAM devices were more accurate earlier in the trials than later and true positive detection rates declined over time of wear. Reduced sensitivity over time may have reflected water accumulation inside the housing. SCRAM correctly detected 57 percent across all BAC events ≥ .02g/dL, with another 22 percent (for a total 79%) detected, but as < .02 g/dL. When subjects dosed themselves to BAC ≥ .08 g/dL, SCRAM correctly detected 88 percent of these events. We conclude that this class of technology does validly detect consumed alcohol at the skin surface, and provides a useful addition to the sentencing options for managing alcohol offenders. The devices are still imperfect and evaluation studies like this one help point toward areas where further development can improve their safety benefit.

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Transdermal alcohol measurement: a review of the literature

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The body of scientific literature on transdermal alcohol testing dates back almost 70 years. The first viable method enabling this knowledge appeared in the 1980s in the form of an alcohol "sweat-patch." Alcohol testing by transdermal (i.e., through the skin) methods is relatively unknown compared to blood, breath, or urine testing. Over the past several years, products that use transdermal alcohol measurement to screen for alcohol consumption and estimate Blood Alcohol Concentration have appeared in the marketplace.

Researchers have performed significant transdermal alcohol measurement research utilizing a number of different research techniques with very consistent results. Based on the published literature, one must conclude that: (1) ethanol is excreted through the skin in sufficient quantities to estimate Blood Alcohol Concentration (BAC); (2) those who have not consumed alcohol do not produce signals that can be interpreted as a transdermal alcohol curve; (3) Transdermal Alcohol Concentration (TAC) is correlated with BAC in both magnitude and shape of the alcohol curve; (4) the TAC alcohol curve is right shifted from the BrAC alcohol curve and takes longer to reach zero; and (5) measuring TAC on a constant basis provides an effective screen for alcohol consumption and an approximation of the magnitude of that consumption. The variability in the kinetics of ethanol transport through the stratum corneum and the variations between peak values of BAC and TAC dictate that today's transdermal devices cannot directly replace a breath analyzer, but can semi-quantitatively identify drinking episodes in a continuous screening environment. Further research and improved modeling techniques of ethanol transport through the skin are required to obtain more quantitatively accurate transdermal results.

Drug impaired drivers: patterns of use

Conducteurs sous l'emprise de stupéfiants: différents modèles

Screening for drugs in oral fluid: illicit drug use and drug driving in a sample of Queensland motorists

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OBJECTIVE: Police Services in a number of Australian states and overseas jurisdictions have begun to implement or consider random road-side drug testing of drivers. This paper outlines research conducted to provide an estimate of the extent of drug driving in a sample of Queensland drivers in regional, rural and metropolitan areas.

DESIGN AND METHODS: Oral fluid samples were collected from 2,657 Queensland motorists who volunteered to participate in the study after proceeding from a Random Breath Test site (RBT). Illicit substances were screened using the Cozart® RapiScan oral fluid drug test device and included cannabis (Δ^9-tetrahydrocannabinol [THC]), amphetamine type substances, heroin and cocaine. Drivers also completed a self-report questionnaire regarding their drug-related driving behaviour.

RESULTS: Overall, 3% of the sample (n = 80) screened positive for at least one illicit substance, although multiple drugs were identified in a sample of 29 respondents. The most common drugs detected in oral fluid were methamphetamine (*n* = 43), cannabis (delta 9 THC) (*n* = 36) followed by amphetamine (*n* = 26). A key finding was that cannabis was confirmed as the most common self-reported drug combined with driving and that individuals who tested positive to any drug through oral fluid analysis were also more likely to report the highest frequency of drug driving. Furthermore, a comparison between drug vs drink driving detection rates for the study revealed a higher detection rate for drug driving (3%) vs drink driving (0.8%).

CONCLUSIONS: This research provides evidence that drug driving is relatively prevalent on Queensland roads, and may in fact be more common than drink driving. The paper will further outline the study findings and present possible directions for future drug driving research.

Roadside detection of drugs in drivers

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Roadside detection of drugs has been conducted in Victoria since late 2004. The drugs targeted have been cannabis (THC), methamphetamine (MA) and more recently methylenedioxymethamphetamine (MDMA). The procedure has been published previously [Drummer, O. H., Gerostamoulos, D., Chu, M., P., S., Boorman, M., Cairns, I., Drugs in oral fluid in randomly selected drivers. Forensic Sci Int. (in press)]. A preliminary test for drugs was conducted on randomly selected drivers stopped at a road block using the DrugWipe II® (Securetec) from a tongue wipe while the driver was still in the vehicle. The presence of a clear positive band for either THC or methamphetamines, or both, resulted in

a second test conducted in a specially designed "Drug Bus" using a specimen of oral fluid (OF) collected by the Cozart Collector. An aliquot of oral fluid collected was tested on the Rapiscan®. The methamphetamines test strip has cross-reactivity to MA and MDMA. Oral fluid on presumptive positive cases was sent to the laboratory for confirmation using GC-MS with limits of quantification of 5, 5 and 2 ng/mL for MA, MDMA and THC, respectively. In cases where oral fluid could not be taken blood was collected and analyzed by similar methods.

There have been almost 30,000 road-side drug tests performed in 2 years of the program. There were 507 oral fluid specimens submitted for confirmation and these gave 414, 228 and 140 cases positive to MA, MDMA and THC, respectively. The median oral fluid concentrations (undiluted) of MA, MDMA and THC were 1194, 2733 and 64 ng/mL. The drug positive rate (to both drug types) has remained largely unchanged since the program started at a little over 2% of screened drivers which was over twice the random breath alcohol rate (legal limit ≤ 0.05%).

There were two false oral fluid positives to cannabis when the results of both on-site devices were considered and ten to methamphetamines, or a false positive rate of 0.04% per screened drivers. However, the false positive rate of the Rapiscan device used alone was 8-fold higher at 0.32% (14 cases for methamphetamines and 85 cases for THC).

There were 70 blood samples submitted. These produced positive drug results in all but one case. Median concentrations for MA, MDMA and THC were 106, 280 and 6 ng/mL, respectively.

The OF false negative rate could not be evaluated since oral fluid is not obtained from screened negative drivers. However, the lowest concentrations of MA and MDMA when only one of these methamphetamines was present and the Rapiscan gave a presumed positive result, was 57 and 66 ng/mL, respectively. The lowest concentration THC in OF was 11 ng/mL from a median of 108 ng/mL. This reinforces the value of using two devices in series in a roadside setting rather than one device alone to achieve a low false positive rate. The data also indicates the high prevalence of methamphetamines and cannabis in persons driving motor vehicles and indicate the continued need to modify driver attitudes to impairing substance use.

Mandatory random roadside drug testing of truck drivers, nightclub patrons and the general driving population in Victoria, Australia

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In December 2003, the Parliament of Victoria passed the Road Safety (Drug Driving) Act 2003 to provide for random drug testing of drivers and to create new offences for failing a drug test. The Act made it illegal to drive with any concentration of methamphetamine or D9-tetrahydrocannabinol present in the blood or oral fluid. Testing commenced on 13 December 2004, with three target drug user groups: truck drivers, nightclub or 'rave party' attendees, and the general driving population. This paper presents the results collected during the first six months of the program as part of a process evaluation conducted to allow reporting to Government before the sunset clause of the legislation expired.

The roadside procedure commenced with random breath testing for alcohol, followed by a preliminary oral fluid test. If positive, a second oral fluid test or blood test was taken. Confirmatory tests on positive roadside samples were conducted in the laboratory. For the evaluation, Victoria Police provided de-identified data on number, location, time and outcomes of random drug tests. Outcomes of the laboratory confirmatory analyses were provided by the Victorian Institute of Forensic Medicine.

Of the 6,657 preliminary oral fluid tests conducted in the first six months, 2.5% were positive to one or both drugs. Laboratory analyses found that 142 oral fluid and blood samples were positive for one or more drugs (2.1% of roadside tests). Drug prevalence was higher in the sessions targeting nightclub attendees (4.7%) than truck drivers (1.6%) or the general public (0.9%). MDMA (ecstasy) was detected in the laboratory analysis of 1.2% of drivers. Sessions targeting nightclub attendees also resulted in a higher prevalence of drivers exceeding the legal blood alcohol limit (0.05% BAC or 0.02% for novice and professional drivers). These drivers were not drug tested, which may have reduced the overall estimates of prevalence of drug driving. These results confirm the findings of previous studies that show nightclub attendees have a high prevalence of drug driving but conflict with other studies that would predict higher levels of methamphetamine among truck drivers.

Saliva as a possible second sample matrix

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Since 1985, the state of New Hampshire (NH) has captured breath samples for reanalysis by defendants in Driving Under the Influence of Alcohol cases. NH collects breath samples on silica gel using the Intoxilyzer 5000EN. Because breath capturing is a blind action and can be influenced by many unseen elements, we are looking for a better representation for second sample analysis.

Saliva is a matrix that is being used more and more to test for drugs as well as alcohol. It has been documented that saliva reflects blood and breath when direct samples

of saliva were tested. For this initial phase we are using "Quantisal[tm], saliva collection device with a volume adequacy indicator" to collect our saliva samples as well as analyzing breath samples on an Intoxilyzer 5000EN. The saliva collection device is a cottony paddle with a plastic handle containing a window which changes from white to blue when enough saliva is trapped in the cotton. There is a slice in the cotton just below the plastic handle. This slice is a focal point weakening the whole paddle structure and causing the paddle to completely separate from handle when wet.
27 samples of saliva and breath were collected from 4 subjects at a controlled drinking session over a 6 hour period. One male subject (#2) – age 41, weight 192 lbs, consumed 4 mixed drinks containing vodka, Kahlua and Bailey's. Three female subjects (#1, #3 and #4) – ages 28, 39 and 48, weight 155 lbs, 170 lbs and 125 lbs respectively. Subject #1 consumed 4 vodka drinks with various mixes. Subject #3 consumed 6 beers and #4 consumed 4 glasses of white wine.
All Intoxilyer (breath) readings were collected prior to the saliva samples and were individually followed by a 0.10 g/210L external standard. The analysis protocol for the saliva samples is the same used for blood alcohol. Saliva samples were analyzed on a Perkin Elmer (PE) Clarus Gas Chromatograph with HS 40 headspace autosampler using PE BAC1 and BAC2 dual capillary column at isothermal conditions (40°C). N-propanol was used as an internal standard.
The mean peak breath value is 0.122 g/210L. The mean peak saliva value was 0.146 g/100mL. The saliva values tended to be about 20% higher than the breath values. Possible contributions to the variance are: saliva/breath partitioning and saliva sampling inconsistencies. For example, the separation of the paddle from the handle caused the indicator to appear too soon and short samples were obtained. The largest deviation between a breath sample and a saliva sample was 0.054.
Future studies will include a larger population of controlled drinking subjects as well as additional collection devices.

Pilot Study for the US National Roadside Survey, 2007

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The preliminary fieldwork for the 2007 National Highway Traffic Safety Administration National Roadside Survey (NRS) was the NRS pilot program conducted in 2005 and 2006. This Pilot Study developed and tested techniques to enhance previous NRS Program methods and included the collection and analysis of oral fluid and blood samples from the nighttime weekend driving population. Breath and oral fluid samples were successfully collected from more than 600 drivers at 6 locations across the U.S. Blood samples were obtained from approximately half of those subjects. Laboratory analyses for alcohol and other drugs were conducted on both the oral fluid and blood samples. Procedures and results from this pilot work formed the basis of the plans for the upcoming 2007 survey.
This pilot program demonstrated a possible relationship between impaired nighttime weekend driving, AUD status, and drug use. The drug results from the 2005 Pilot Study indicated 15% of the nighttime weekend driving population tested positive for drug use and 9.4% tested positive for alcohol based on blood and oral fluid testing. Summarized, 22.7% of all drivers were positive for drugs and/or alcohol. This preliminary evidence based on a relatively small sample size forms the basis for the proposed effort that seeks to expand the scope of work carried out in the pilot, to include augmentation of the upcoming 2007 NRS. This large-scale study offers the unique opportunity to measure the prevalence of ATOD use using multiple methods. Data collection for the upcoming NRS is scheduled to begin in July 2007 and will culminate in the collection of data from at least 100 weekend nighttime drivers at 60 sites nationwide (n=6,000 nighttime drivers) and 1,500 daytime drivers. This presentation will highlight the salient features of the data collection methodology and discuss the preliminary drug and alcohol results from the pilot study.

Plans for the US National Roadside Survey, 2007

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In the summer and fall of 2007, a US national effort will be mounted to survey over 6,000 weekend nighttime and 1500 daytime motorists in the U.S. to determine the prevalence of alcohol and drug use by drivers and the proportion of this population that can be identified as having alcohol use disorders and drug use disorders. This is the fourth in the series of National Roadside Surveys that have been conducted every decade since 1973. However, this is the first to take advantage of the emerging technology of oral fluid analysis to collect information on drug use by drivers and it will be the first to conduct interviews to identify drivers with DSM IV alcohol and drug use disorders. Thus, this will be the first time that a full picture of AOD use and resulting problems will be available for vehicle operators on the road during high risk weekend periods. This talk will describe the plans for the survey, which will for the first

time include a sample of daytime drivers to clarify drug and alcohol impaired driving in this population. The project is funded and sponsored by the National Highway Traffic Safety Administration with the assistance of the National Institute on Alcohol Abuse and Alcoholism, the National Institute on Drug Abuse and the National Institute of Justice.
Based on the data to be collected we will for the first time be able to extensively relate measured BACs and drug assays of and both oral fluid and blood of drivers with their self-reported drug use and their reported lifetime and recent alcohol and drug consumption levels and drinking and drug problems. The survey will shed considerable light on the extent to which DSM IV dependence and abuse indicators are associated with driving, measured blood alcohol concentration and measured blood drug concentration.

Correlation of drug profiles in paired blood and saliva samples from randomly selected drivers: implications for the US National Roadside Survey 2007

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One of the aims of the analysis of paired blood and oral fluid specimens in randomly selected night-time drivers was to determine the possible correlation between drug profile and drug concentration in the two matrices. The specimens were collected at six different locations within the USA, then shipped overnight to the laboratory for analysis. The laboratory was completely blinded to the pairing system, and the data were provided back to the study group as individual specimen results. All samples were tested for ethanol as well as a wide range of drugs: cocaine and metabolites, amphetamines (including MDMA, MDA and MDEA), opiates (including oxycodone), phencyclidine, cannabinoids, benzodiazepines, tricyclic antidepressants, methadone, methylphenidate, sertraline, fluoxetine, barbiturates, tramadol, zolpidem and carisoprodol.
In total, 639 oral fluid and 394 blood samples were collected. All subjects providing a blood sample also provided an oral fluid, while all subjects providing an oral fluid specimen did not necessarily consent to blood collection. Thirty-three (33) pairs of samples were positive and the results correlated very well. The main discrepancy was in the case of benzodiazepines, which in general have low saliva: plasma ratio. Three blood samples were positive for the class, while the corresponding oral fluid samples were not. For cannabinoids, oral fluid detected four more cases than blood for the active component, THC. When the metabolites THCA and 11-OH-THC were included in the confirmation profile for blood, three of those four were identified. However, the presence of the parent drug is significant in forensic analysis.

Drug Class	Oral fluid positive	Blood positive
Tricyclic antidepressants	1	1
Amphetamines	5	5
Carisoprodol	1	1
Cocaine and metabolites	4	4
Fluoxetine	4	4
Hydrocodone	2	2
Oxycodone	1	0
Sertraline	5	5
Tramadol	2	2
Benzodiazepines	0	3
Tetrahydrocannabinol (THC)	15	11
THCA and /or 11-OH THC	Not tested	+ 3 = 14

Note: Some samples had multiple positive results

In the on-going 2007 study, additional drugs to be tested include meperidine, ketamine, dextromethorphan and propoxyphene. In addition, oral fluid will be tested for the marijuana metabolite, THCA as has been recently published.

POSTERS PRESENTATIONS
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Analytical advances
Développements récents en analyse

Liquid Chromatography - Tandem Mass Spectrometry in Forensic Toxicology

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AIMS: This paper reviews our studies, applications and advances of liquid chromatography-tandem mass spectrometry (LC-MS/MS) in forensic toxicology.

METHODS: LC analysis was performed using a Agilent 1100 HPLC system coupled to API 4000 triple quadrupole mass spectrometer. A series of simple, sensitive and rapid methods were developed for the screening and identification of compounds of interest in blood, urine, oral fluid, and hair, covering opiates, cocaine, amphetamines, cannabinoids, LSD, antidepressants, benzodiazepines, barbitals, hypnotics, neuroleptics, pesticides (organophosphorus, carbamates), antiepileptics, b-blockers, anabolics, corticosterioids, rodenticides, paraquat, poisonous alkaloids, tetrodotoxin, and some clinical drugs.

RESULTS: The developed analytical methods were successfully applied in the fields of postmortem toxicology, clinical toxicology, driving under the influence of drugs of abuse, and drug-facilitated crime. For example, determination of 6-acetylmorphine, morphine, and codeine in head hair; determination of MDMA and ketamine in the hair segments from a pregnant woman and her infant; analysis of benzodiazepines from patients admitted to the emergency hospital; detection of paraquat, aconitine, or tetrodotoxin in fatal poisoning cases. Data acquisition under MS/MS was achieved by applying multiple reaction monitoring of two fragment ion transitions to provide a high degree of sensitivity and selectivity for both quantification and confirmation. On the other hand, some problems we confronted were discussed, including sample preparation, matrix effects, chromatographic separation, the volume injected, contamination, and identification criteria.

CONCLUSIONS: Although some drawbacks still remain in establishing LC-MS/MS procedures, it is clear that LC-MS/MS provides a valuable alternative to GC-MS in forensic toxicology. LC-MS/MS may become the gold standard in clinical and forensic toxicology.

Development of a System Suitability Test for Forensic Toxicology Applications of LC-MS/MS

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AIMS: When evaluating the overall performance of an LC-MS/MS system, it is necessary to design a test that will assess each component: LC pump, autosampler, column, and mass spectrometer. Analytes need to be selected so they span a large polarity range, therefore retention time range, and the compounds should also cover the mass range of interest. If full scan MS/MS is utilized, the fragmentation capabilities of the instrument must also be evaluated. The objective of this presentation is to identify the important criteria to consider when evaluating LC-MS/MS system performance. As LC-MS/MS is increasingly used in routine forensic analyses, a quick, effective system suitability test is necessary to evaluate the system performance and troubleshoot any potential problems.

METHODS: Systems consisted of various LC stacks interfaced to hybrid triple quadrupole/linear ion trap (QQQ/LIT) mass spectrometers. Hybrid QQQ/LIT instruments were used so that both MRM and full scan MS/MS capabilities could be evaluated. The analytes used were caffeine, morphine, codeine, haloperidol, amiodarone, methamphetamine, doxepin and diazepam. Mobile phases were: A) 1 mM ammonium formate and B) 95:5 acetonitrile: 1 mM ammonium formate with 0.1% formic acid added to each. An Applied Biosystems pentafluorophenyl column (2.1 mm x 50 mm) was used for separation. These conditions were chosen because they are relatively generic. The gradient was started at 10% B and ramped to 90% B over 10 minutes, where it was held for 0.5 minutes then dropped to the initial starting composition and equilibrated for 2.5 minutes. Total run time was 15 min. This test was designed to check the performance only in ESI+ mode. MRM transitions and their optimal collision energies for each analyte were determined using the autotune/quantitative optimization feature of the mass spectrometer. Two transitions per analyte were monitored.

RESULTS AND CONCLUSIONS: Several analytes were used to evaluate the performance of several LC-MS/MS systems. Morphine and caffeine were chosen as a polar, relatively unretained analytes, and amadiodarone was used as a relatively non-polar, late eluting analyte. Haloperidol exhibited a fragmentation pattern that was useful for evaluating the fragmentation performance of the mass spectrometer. Inter- and intra-day reproducibility of retention times, peak intensities, and peak area ratios were recorded and monitored to gauge instrument performance over time. A change in any of these parameters, such as peak shape or intensity, could indicate hardware problems, e.g. pumping problems or a dirty instrument. By using a test mixture that includes analytes over wide polarity and mass ranges, system performance can be checked and monitored to identify any problems before case samples are run.

ESI-MS/MS Library of 1,250 Drugs: Database for Drug Identification with Triple-Quadrupole-MS and Qtrap Instruments

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AIMS: A tandem-mass spectra library with electrospray mass spectrometry of 1250 compounds has been developed using a QTrap tandem-mass spectrometer with Linear Ion Trap (LIT) technology (Applied Biosystems/Sciex) with a TurboIonSpray source.

METHODS: Spectra were obtained at three different collision energies and collision energy spread (CES) with positive and/or negative ionization. The novel aspects compared to our former library [1,2] produced with a standard triple-quadrupole MS (API 365) is the enlargement of the ESI-MS/MS library and the use of collision energy spread applicable for fast multi-target screening (MTS) [3] with enhanced product ion scan mode. For setting up the library, 1 ng to 2 µg of compounds were injected and product ion spectra of the

precursor ions were generated by CID in the collision cell using three different collision energies (20, 35 and 50 eV, respectively) and collision energy spread (35 ± 15 eV). The instrument was calibrated with polypropylene glycol (mass accuracy and resolution) before acquisition of a series of compounds and haloperidol and glafenine were used for quality control of the spectra after ten injections for positive and negative ionization, respectively. The spectra have been added in the Microsoft Access database, which is generated by the Analyst software.

RESULTS: The library contains over 5,600 spectra of 1,250 forensically and clinically important drugs, such as illegal drugs and some deuterated analogues, hypnotics, amphetamines, benzodiazepines, antidepressants, neuroleptics and many others. It also contains CAS number, compound class, molecular formula and molecular structure of each compound.

CONCLUSIONS: The new library with over 1,250 compounds can be used for identification of MS/MS spectra obtained at 20, 35 and 50 eV collision energy as well as collision energy spread spectra at 35 ± 15 eV. It is also a useful tool for setting up individual multi target procedures for screening or quantitative analysis using Multiple Reaction Monitoring.

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A Novel, Turnkey MS Replacement Strategy for Traditional LC/UV Drug Screening Technology

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AIMS: The ratio of mass spectrometry experts to non-experts has been declining over the years, as LC-MS/MS finds new applications over a broad range of markets, such as food testing, environmental analysis, forensics, etc. An obvious response to this problem is to reduce the complexity level of the user interface to the system, and thereby reduce the expertise level required by the user. Such a solution works only if the acquisition and processing know-how is built into the product. This poster presents a browser-like software integrating LC-MS/MS hardware and methodology in a turnkey solution for novice and expert users alike.

METHODS: An example of an application where LC-MS/MS is set to displace a traditional technique is forensic drug screening. For the last 20 years, the technique of choice for rapid screening has been LC/UV with automated on-line sample prep, and reporting, which reduces the analysis to a very simple operation. Replacing this type of technique with LC-MS/MS presents a challenge in the design of the software and development of the acquisition and processing methods. A multiple reaction monitoring (MRM) as survey scan and an enhanced product ion (EPI) scan as dependent scan were performed in an information-dependent acquisition (IDA) experiment. Finally, drug identification was carried out by library search with a newly developed MS/MS library based on EPI spectra.

PRELIMINARTY RESULTS: This paper presents the results of a development project to create a drop-in replacement LC-MS/MS solution for drug screening LC-MS/MS users. The system performance characteristics and examples are provided, along with a back-to-back comparison with traditional LC/UV techniques. Cliquid™ Software provided an ideal platform for development of an easy-to-use turnkey solution. Combined with the appropriate LC-MS hardware, the novel browser-like interface allows users of the existing LC/UV techniques to transition to more advanced LC-MS/MS technology with very little training, and very little change to the existing sample/data handling procedures. The methods included with the system utilize the unique scan capabilities of the 3200 Q Trap® hybrid triple quadrupole linear ion trap LC-MS/MS systems to provide rapid, simultaneous screening and identification. Combined with an included 1,200 compounds LC-MS/MS library and a variety of automated reporting options, this novel solution provides a drop-in replacement for existing LC/UV techniques for drug screening applications.

Fast Gas Chromatography/Mass Spectrometry in Toxicological Analyses: Definition, Essential Parameters and Applicability

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AIMS: A majority of toxicology laboratories are performing high volume batch-to-batch analyses for screening and/or confirmation of different drugs. For various applications, gas chromatography/mass spectrometry (GC-MS) is still the method of choice of many laboratories owing to versatile, cost-efficient and reliable analytical performance. Analytical methodology published is generally based on conventional GC

separation. However, fast GC might provide marked increments in sample throughput, decreasing analyses costs and GC-MS instrumentation needed, while still maintaining sufficient chromatographic resolution and method performance.

METHODS: A short overview of the most important parameters for an analyst to apply fast GC-MS is provided, such as a choice of GC carrier gas, above-optimum carrier gas flow, high initial and final temperature, column dimensions, MS data acquisition, high temperature ramping and cooling of GC oven. Examples of fast GC-MS –based methodology in an area of drugs of abuse analyses, such as for amphetamines and benzodiazepines, using commercially available bench-top GC-MS instrumentation is presented and compared with conventional GC-MS from practical perspective and routine applicability.

RESULTS: Chromatographic runtimes of typically 15-20 min by conventional GC-MS, are shown to be reduced from 2-5 min by applied fast GC-MS systems also in validated multicomponent analyses. The most significant speed gains in analysis time are generally achieved by fast temperature ramping of GC oven and reducing column dimensions. Nevertheless, optimization of other GC-MS parameters is also mandatory and might well lead to significant further reductions in GC separation time while simultaneously maintaining sufficient sensitivity, sample capacity and resolution of the assay. Moreover, instrumental limitations, especially in terms of insufficient temperature ramping capability of GC oven and too slow quadrupole MS detection speed, prevent to put into a practice certain applications.

CONCLUSIONS: In conclusion, careful optimization of sample capacity, sensitivity, speed and resolution of GC separation generally pays the price and in combination with fast GC-MS parameters is a powerful tool in toxicology. It should also be noted that in many applications using fast GC-MS, modern, but standard bench-top GC-MS system can be used without any further instrumental modifications. More laboratories are encouraged to take advantage of fast GC-MS instead of conventional GC-MS, which should lead to more cost-effective, but still reliable analytical solutions, if properly optimized.

Solid Phase Extraction (SPE) of Illicit Drugs – Can a Single Sorbent Cater to Acidic, Neutral and Basic Drugs in Biological Matrices?

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AIMS: Illicit drugs encountered in forensic and toxicological laboratories consist of acidic, neutral and basic compounds of widely different polarities. To isolate these drugs from biological matrices such as urine, plasma and whole blood, solid phase extraction (SPE) is commonly used. Documented literature indicates the use of a variety of silica and polymer based sorbents with hydrophobic and cation exchange interaction characteristics for eliminating proteinaceous and other organic/inorganic contaminants for SPE-based clean up of biological samples prior to GC/MS or LC/MS analysis. However, sorbent selection still remains a dilemma. In this presentation, we explore three silica-based and two polymeric sorbents for the purification of (1) THC and its metabolite THC-carboxylic acid, (2) Cocaine and its metabolites benzoylecgonine (BE) and cocaethylene (CE) and (3) Diazepam and related benzodiazepine drugs. Both GC/MS and LC/MS were used for analysis.

METHODS: Urine samples containing THC, THC-COOH, cocaine, BE, CE, diazepam, lorazepam and temazepam (15-100 ng/mL each), were extracted through Strata-X-C, -Screen-C, -X, C-18E and C-8 cartridges (30-100 mg/mL) using a variety of washes and elutions.

RESULTS: Strata-X-C is a styrene-divinylbenzene polymer with sulfonic acid functionalities and is a strong cation exchange sorbent. It retains THC and THC-COOH, as well as some of the benzodiazepines, even with a 30% acetonitrile or 50% methanol wash and elution with methanol furnishes very clean extracts. Cocaine and its metabolites and some benzodiazepines are eluted from this sorbent with 5% ammonium hydroxide in methanol. For all drugs, recoveries are greater than 90%. Similar results are obtained with the mixed mode silica-based strata-Screen C (hydrophobic and strong cation exchanger), but for hydrophobic molecules like THC and THC-COOH, a less stronger organic wash (20% methanol) had to be used for maximizing recoveries. For neutral sorbents such as the silica based C18 or C8, only a less stringent organic wash can be performed, which resulted in incomplete elimination of contaminants for all the drugs tested. The polymeric polar neutral sorbent strata-X could sustain a stronger organic wash for THC and THC-COOH, but for benzodiazepines, only a milder wash could be used. From this comparative evaluation of silica and polymeric SPE sorbents, it emerges that the polymeric strong cation exchanger strata-X-C is the most versatile for all kinds of drugs in furnishing cleaner extracts along with excellent recovery yields. However, caution should be exercised in the case of nitrogen containing compounds, since factors like pKa (acidity) and structural features play a significant role in determining whether such drugs would elute in the methanol fraction or under basic elution conditions. For example, Diazepam elutes from strata-X-C only with methanol containing 5% ammonium hydroxide, while Lorazepam and Temazepam come down in methanol. On the other hand, the strongly basic Cocaine and metabolites need basified methanol elution.

CONCLUSIONS: Overall, strata-X-C appears to be closest to a universal solution for the SPE-based sample purification of drugs in biological matrices.

Simple and Simultaneous Determination of Twelve Phenothiazines in Human Serum by Reversed-Phase High-Performance Liquid Chromatography

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AIMS: The aim of this presentation is to describe an HPLC-UV method for the simultaneous determination of 12 phenothiazines in human serum.

METHODS: Serum (1 mL) was extracted with 3 mL t-butyl ether after the addition of 200 µL 1N NaOH and 40 µL diazepam (10 µg/mL, IS). After mixing and centrifugation the organic phase was transferred and evaporated to dryness. The residue was reconstituted in 200 µL mobile phase and 50 µL injected into the HPLC equipped with a C18 column (250 mm x 4.6 mm I.D., particle size 5 µm, Inersil ODS-SP). The mobile phase, consisting of acetonitrile : methanol : 30 mM NaH_2PO_4 (pH 5.6) (300:200:500, v/v/v), was delivered at a flow rate of 0.9 mL/min, and detection was at 250 nm.

RESULTS: The 12 phenothiazines assayed are listed with their retention times (min) and limits of quantitation (ng/mL), respectively: propericiazine (11.3, 3.7), promethazine (15.3, 3.2), profenamine (16.2, 3.5), levomepromazine (17.8, 4.5), thioproperazine (19.3, 4.1), perazine (21.1, 4.6), chlorpromazine (22.3, 3.6), IS (25.7, not applicable), perphenazine (27.5, 4.0), thioridazine (31.7, 4.1), fluphenazine (39.6, 5.5), prochlorperazine (43.2, 4.9) and trifluoperazine (62.0, 5.2). The LOQ was defined as the lowest concentration on the standard curve that could be measured with acceptable accuracy and precision: No interfering peaks appeared when the following drugs were added to serum lofepramine, theophylline, caffeine, thiamyral, phenobarbital, carbamazepine, desipramine, estazolam, nitrazepam, oxazepam, dosulepin, imipramine, triazolam, flunitrazepam, etizolam, deorodone, midazolam and haloxazolam.

The inter-day reproducibility was assessed using six samples at two different concentrations (100 and 200 ng/mL) in analyzed in triplicate on the same day. The CVs ranged from 1.2 - 6.3%; the accuracy was found to be in the range of 95.7 - 102.1%. The intra-day reproducibility was determined using two different quality control samples over a two-week period. The CVs ranged from 2.7 - 6.3%; the accuracy was found to be in the range of 95.5 - 102.1%. The calibration curves for the 12 drugs were linear over the concentration range of 2 - 500 ng/mL (r^2 = 0.996 - 0.998). A stability study demonstrated the drugs and the IS were stable in serum up to 8 h at 20°C and up to 2 weeks when stored at 4°C and –20°C. Therefore, extracted samples were stored refrigerated at 4°C for same-day analysis and serum samples were frozen at –20°C until analysis.

CONCLUSIONS: This sensitive and selective method allows for simultaneous screening and quantification of almost all phenothiazines available in Japan for the purposes of clinical and forensic applications.

Simultaneous Determination of Amphetamine Related Compounds in Human Plasma by HPLC with Peroxyoxalate Chemiluminescence Detection

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AIMS: Abuse of amphetamine related compounds (APs) such as methamphetamine (MP), amphetamine (AP), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and p-methoxymethamphetamine (PMMA) has been spreading and causing serious social problems worldwide. Recently, it was found that illegal MDMA tablets contain several abused drugs, and also multi-drug use become popular. Thus the adverse reactions may be caused due to the unexpected interaction among the concomitant drugs. To protect human health from these risks, a sensitive and selective analytical method for simultaneous determination of drugs of abuse is requisite. In this study, an HPLC-peroxyoxalate chemiluminescence (PO-CL) detection method for above APs including hydroxylated metabolites, p-hydroxymethamphetamine (p-HMP) and p-hydroxyamphetamine (p-HAP), in human plasma was examined. Sensitive determination of APs could be achieved by a fluorescence labeling with 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F).

METHODS: APs in human plasma were extracted with ethyl acetate. The extracts were evaporated under nitrogen gas, and labeled with 20 mM DBD-F in borate buffer (pH 8.5) for 20 min at 80°C. The DBD-derivatives were isocratically separated within 45 min by an ODS column (150 x 4.6 mm, 3 µm) with a mixture of 10 mM imidazole-HNO_3 buffer (pH 6.5) : acetonitrile : tetrahydrofuran (52:44:4, v/v/v%) at a flow rate of 0.7 mL/min. The post-column PO-CL reagent

solution use was a mixture of 2 mM bis (2,4,5-trichloro-6-carbopentoxyphenyl) oxalate and 15 mM hydrogen peroxide in acetonitrile.

RESULTS: The calibration curves of APs and hydroxylated metabolites were linear with more than 0.995 of correlation coefficiencies. Detection limits (S/N=3) of MDMA, MDA, MP, AP, PMMA, p-HMP and p-HAP were 0.10, 0.75, 0.08, 1.10, 0.06, 0.14 and 0.75 ng/mL, respectively.

CONCLUSIONS: The proposed method is sensitive enough to determine lower concentrations of APs in human plasma, and thus it might be applicable for their forensic or toxicological study.

Rapid Diagnosis of a Drug Intoxication Using Novel GC/MS Software NAGINATA™

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AIMS: In order to improve the reliability and time for data-analysis in drug screening, we developed a new screening procedure using GC-MS software NAGINATA™.

METHODS: Fifty abused drugs were selected as target drugs, which include methamphetamine, methamphetamine-, tryptamine-, phenethylamine-derivatives, opiates and benzodiazepines. The drugs were analyzed by gas chromatography-mass spectrometry using a retention time lock after solid-phase extraction with a Focus™ column and acetylation. Based on the results obtained, a database of "abused drugs" was constructed from the parameters of each drug including retention time (RT), qualifier ion/target ion (QT) percentage and calibration curve (value of slope and intercept) using a GC-MS software package – NAGINATA™. This add-on software recently released by Nishikawa Keisoku Co., Ltd (Tokyo, Japan) is designed for the evaluation of quality control in an Agilent GC-MS system (5973/75MSD) with automatic data analysis by using a constructed database. Triage™-positive urine samples from forensic cases were analyzed by the method described above, and data analyses were performed by this software using the newly constructed database.

RESULTS: We found a significant improvement in the time needed for data-analysis. Reliable drug confirmation and a rough estimation of drug concentration were achieved simultaneously. Furthermore, once the database was constructed, standards were not required for subsequent analyses of case samples.

CONCLUSIONS: This new comprehensive screening procedure using NAGINATA™ offers a method for the rapid diagnosis of poisoning, and it should be useful in clinical and forensic toxicological examinations.

A Fast and Sensitive Screening Method for Buprenorphine and Norbuprenorphine in Urine and Whole Blood by Solid Phase Extraction and Ultra High Performance Liquid Chromatography/Time-Of-Flight Mass Spectrometry (UPLC/TOFMS)

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AIMS: Buprenorphine (i.e. Subutex®, Suboxone® or Temgesic®) is an emerging drug in substitution therapy of opioid addicts in Denmark. Screening for buprenorphine by radioimmunoassay (RIA) has previously been applied in our laboratory, but the buprenorphine RIA kit is no longer available. Thus, the objective of this study was to develop a sensitive, fast and accurate screening method for buprenorphine.

METHODS: Buprenorphine (MW 468.3114) and norbuprenorphine (MW 414.2644) were extracted by mixed mode cat ion exchange columns, Isolute HCX-3 (130 mg/3 mL), by a modified method (1). Internal standards D4-buprenorphine (MW 472.3365) and D3-norbuprenorphine (MW 417.2833) were added to all samples prior to extraction. Urine (2 mL) was added 1 mL 1 M acetate buffer, pH 5.5 and 25 µL beta-glucuronidase/ arylsulphatase and hydrolyzed overnight at 40°C. Whole blood (0.5 mL) was added 2.7 mL 0.1 M KH2PO4 buffer, pH 6, mixed, sonicated 10 min and centrifuged 10 min at 3600 rpm. Gilson ASPEC XL4 (Gilson, Viliers-le-Bel, France) with positive pressure adjustment (Biolab, Aarhus, Denmark) was used for automated solid-phase extraction. The SPE columns were conditioned with 1.5 mL methanol and 1.5 mL 0.1 M KH_2PO_4 buffer. Sample (3 mL) was loaded, followed by washing steps consisting of 1 mL 0.1 M KH_2PO_4 buffer, 1 mL 1 M acetic acid, and 1 mL methanol in consecutive order. After drying 10 min, the analytes were eluted with ammonium hydroxide (25% aq.) -acetonitrile -ethyl acetate (2:10:88, v/v). After evaporation at 45°C, the blood extract was reconstituted in 100 µL methanol and the urine extract was reconstituted in 2 mL methanol. The UPLC/TOF/MS conditions: UPLC (Acquity, Waters, Milford, MA, USA) 1.5 min gradient elution with water-formic acid (0.1%) and acetonitrile (75:25) to (50:50) on an Acquity BEH C18, 2.1 x100 mm column, 1.7 µm at 50°C. The TOFMS (LCT Premier XE, Micromass, Manchester, UK) was operated in electropositive W-mode (ESI+).

RESULTS: The retention time was 0.85 min for norbuprenorphine and 1.23 min for buprenorphine, and the total UPLC run time was 3 minutes. The mass accuracy was found to be better than 5 ppm and resolution higher than 10,000. The linear range of the instrument method was 0.00005 to 0.1 mg/L for buprenorphine

and 0.0005 to 0.1 mg/l for norbuprenorphine. Accuracy was verified by including spiked samples in each run. The limit of detection (LOD) in blank matrix was found to be 0.0001 mg/L in blood and 0.0005 mg/L in urine for buprenorphine, and 0.001 mg/L blood and 0.005 mg/L urine for norbuprenorphine. Thus, the LOD of buprenorphine in blood was found to be adequate for detection of buprenorphine in the therapeutic range; 0.0001 to 0.001 mg/L.
Urine from authentic criminal and autopsy cases were applied for method comparison; 18 positive and 5 negative RIA-buprenorphine urine samples were confirmed by UPLC/TOFMS.

CONCLUSIONS: A UPLC/TOFMS method for fast separation and accurate detection of buprenorphine and norbuprenorphine was obtained.

Reference:

1. H. Klinke and K. Linnet: Performance of four mixed-mode solid-phase extraction columns applied to basic drugs in urine. The Scandinavian Journal of Clinical & Laboratory Investigation (In Press)

A Semi-Quantitative General Unknown Screening Method for Drugs and Toxic Compounds in Urine Using Liquid Chromatography–Mass Spectrometry

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AIMS: Many clinical and forensics laboratories utilize general unknown screening to identify analytes present in biological samples. Often, once an analyte is identified, laboratories use a second method for quantitation. The aim of this work was to develop one method on a triple quadrupole LC-MS system that could be used to both identify unknown analytes in human urine and determine their concentration semi-quantitatively. Clinicians under time constraints to screen samples such as those in an emergency room setting, could potentially benefit from such a method.

METHODS: An MS/MS spectral library of 300 compounds most commonly observed in toxicology laboratories was created. Quantitation curves were developed for 50 analytes in SPE-prepped human urine utilizing dual mode Hypersep -Verify CX cartridges developed for basic, neutral and acidic compounds. A 13 minute LC method was implemented and samples were analyzed using electrospray ionization on a TSQ Quantum Access triple quadrupole mass spectrometer equipped with a 50 x 2.1 5 µm Hypersil Gold PFP column using ACN and 10 mM ammonium formate in 0.1% formic acid in a gradient mode with a flow rate of 200 uL/min. A scan dependent SRM scan was used for quantitation, followed by scan dependent MS/MS scans for screening against the spectral library.

RESULTS: The screening of 300 compounds was validated by processing and analyzing urine samples spiked with 10 randomly selected compounds in concentrations of 1ng/mL, 10 ng/mL, 100 ng/mL and 1000 ng/mL for all 300 analytes. Data for these representative compounds with m/z of parent ion ranging from 221.2 – 480.2, shown below in Table 1:

Table 1: Data On 10 Compounds

Compound	LOD (ng/mL urine)	LOQ (ng/mL urine)	R^2
Ondansetron	1	1	1
Noscapine	1	1	0.99
Phenyltoloxamine	1	1	0.99
Promethazine	1	1	0.99
Prilocaine	1	1	1
Oxcarbazepine	1	1	0.99
Prazosin	1	1	0.99
Propafenone	1	1	0.99
Buspirone	1	1	0.99
Nicardipine	1	1	0.99

The recoveries from SPE were estimated for all compounds. Calibration curves for 50 compounds were obtained from SPE spiked urine samples and LOQ's were reported for the 50 analytes. Validation results demonstrate a sensitive general unknown screen for 300 compounds, which can semi-quantitate on at least 50 analytes. The method was confirmed in patient urine samples and compared to an established general unknown method on an LXQ ion trap mass spectrometer.

CONCLUSIONS: With a 13 min LC method, automated data processing, and 1 hr for sample preparation (can be shortened with further sample preparation automation) the data turnaround time is less then 2 hours.

The Analysis of Basic Drugs by LC-MS/MS in High pH Mobile Phases

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AIMS: Performing chromatographic separations of basic compounds in high pH mobile phases results in extended retention, excellent peak shapes and good efficiency. One possible drawback could be a severe decrease in sensitivity with MS detection, under conditions that suppress analyte ionization in solution. It is common practice to employ volatile weak acids or low pH buffers for enhancing the ionization of basic compounds in positive ionization mode electrospray (ES^+) LC-MS. Thus the LC-MS analysis of basic drug compounds in mobile phases of high pH (>analyte pK_a^{+2}) could be compromised if ES gas-phase ionization yields were closely linked to acid-base equilibrium in solution.

The aim of this work was to study the effect of pH on ES+ LC-MS/MS sensitivity for basic drug compounds.

METHODS: The signal intensity for various basic drug compounds in standard acidic mobile phase conditions of 0.1% formic acid with acetonitrile were compared to intensities observed in 10mM ammonium bicarbonate buffers of different pH (7.8 - 11), with acetonitrile, as mobile phase components. Analysis was preformed using an Agilent 1100 and API 3000 LC-MS/MS operated in positive ion mode. Separation was done using a Gemini™ 5µm C18 150 mm x 3.0 mm ID column. The feasibility of quantitating various basic compounds in ES^+ LC-MS/MS in high pH mobile phases was evaluated. The limit of quantitation (LOQ), taken to be the minimum analyte quantity on-column giving a S/N of 10, was evaluated. Response linearity was studied in the concentration range 0.05 - 100 ng/mL for most compounds. Method precision was determined by replicate analyses (n = 6) at four concentration levels: 0.05, 0.25, 1.00 and 100 ng/mL. Method accuracy was evaluated by comparing the mean value for six replicate experimental results with the expected value at various concentration levels.

RESULTS: Contrary to common expectations, high pH mobile phases do NOT suppress the ionization of basic compounds in ES^+; positive ions are formed abundantly, and analyte responses are comparable, or most often better in high compared to low pH mobile phases. Most basic compounds included in this study were successfully quantified at the 1.25 pg on-column level, or better, with some at levels as low as 50 fg. A comparison of S/N ratios at very low concentration levels (50 - 100 pg/mL) reveals that most basic compounds included in this study can be detected with better sensitivity in high pH mobile phases. LOQs for some weakly basic compounds such as Trimethoprim, Triamterene, Buspirone and Nizatidine were fairly high (> 250 fg on-column), but still better compared to low pH (except for Buspirone). The linear regression data (R^2) for all 22 basic compounds demonstrated good linearity in all cases with 0.997 in a wide dynamic range (> 103). Method precision was < 14% RSD at low, and < 8% RSD at high concentration levels. Intra-assay accuracy was 92 - 118% at low concentration levels, and 81 - 120% at high concentration levels.

CONCLUSIONS: The successful quantitation of these basic compounds by ES^+ LC-MS/MS in a mobile phase containing a high pH buffer was achieved. This finding is significant as it extends the applicability of generic elution methods to the analysis of polar basic compounds, previously difficult to retain by RP chromatography, without compromising the ability to detect them by mass-spectrometry.

Chemiluminescent Detection of Buprenorphine and Naltrexone with Acidic Potassium Permanganate

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AIMS: Buprenorphine and naltrexone are used in chemical based opiate addiction treatments. In recent years there has been a trend towards using sustained-released implants, rather than oral formulations. This new method of treatment requires a rapid and sensitive analysis to determine therapeutic concentrations of the analytes in biological samples to ensure the dosage rate of the implants is appropriate. One rapid and sensitive detection method is the use of a chemiluminescence reaction with acidic potassium permanganate in the presence of polyphosphates. The literature reports that compounds must contain tertiary amine and/or phenolic functional groups in order to produce a chemiluminescence reaction with permanganate in the presence of polyphosphates. Since both buprenorphine and naltrexone contain these functional groups, investigations were undertaken to determine whether or not a chemiluminescence reaction will occur with these analytes.

METHODS: Studies were performed by continuous flow analysis using a Carey Eclipse fluorescence spectrometer set on bioluminescence mode. The two line flow system utilised a Gilson Miniplus 3 peristaltic pump with 3 mm I.D. Tygon tubing. The analyte was mixed with the reagent at a T-piece 3 cm from the detector and the mixture line was coiled loosely in front of the photomultiplier tube (PMT) window. The following parameters were used: scan range 600 – 800 nm; slit width 20 nm; PMT 1000 volts; permanganate concentration $5x10^{-4}$ M in 1% (w/v) polyphosphate, pH 2; analyte concentration $1x10^{-4}$ M in 1% (w/v) polyphosphate, pH 2; combined flow rate of 6 mL/minute.

RESULTS: Chemiluminescence was observed for both compounds showing a maximum wavelength (λmax) at 655 nm which is the same for all other reported permanganate chemiluminescence reactions in the presence of polyphosphates. The limits of detection (S/N=3) for both drugs were estimated as buprenorphine: 0.16 mg L^{-1}; and naltrexone: 1.09 mg L^{-1} using the described Carey Eclipse fluorescence spectrometer. These detection limits have been improved to 0.01 mg L^{-1} for naltrexone using a purpose built chemiluminescence detector, further improvements can be expected with optimisation of the system.

CONCLUSIONS: The results show that both buprenorphine and naltrexone undergo chemilu–

minescence reactions with acidic potassium permanganate in the presence of polyphosphates. The reaction is such that its sensitivity should allow for its use as a post column method of detection for analysis by high performance liquid chromatography.

Rapid and Sensitive Determination of Nicotine in Biological Fluids and Products using Micellar Liquid Chromatography with Electrochemical Detection

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AIMS: Nicotine is one of the most heavily used addictive substances available. Pharmacologically, nicotine is a compound that acts on the central nervous system to elevate mood. Chronic use of nicotine has serious health consequences including cardiovascular and respiratory disorders including lung cancer. Due to efforts by various governmental agencies in raising awareness, many people are trying to quit smoking through the use of pharmaceutical products such as nicotine patches and gum. The purpose of this work was to develop a micellar liquid chromatography (MLC) procedure for rapid screening and determination of nicotine in cigarettes, pharmaceuticals, serum and urine samples using a hybrid SDS-modifier mobile phase with electrochemical detection and direct sample injection. This simplifies the determination of nicotine in the desired matrix. This method can be useful to analyze nicotine in numerous tobacco and pharmaceutical products, for the clinical monitoring of patients in treatment for nicotine addiction and in forensic cases.

METHODS: MLC is a technique, which uses a mobile phase containing surfactant concentration above its critical micelle concentration (cmc). It is an alternative method to aqueous organic HPLC because of the large number of interactions of solutes with the mobile and stationary phase. The solubilizing ability of micelles is one of their most important properties and allows direct injection of untreated samples. Experimental work was focused on the optimization of the conditions for the simple and rapid, as well as low cost analysis including the selection of the best mobile phase to obtain satisfactory results. The optimization of the method was carried out by using different mobile phases containing sodium dodecyl sulphate as surfactant and propanol, butanol, pentanol as organic modifiers w ith electrochemical detection. Optimal conditions for the detection of nicotine were found using a C18 column, a mobile phase containing sodium dodecyl sulphate 0.15 M-6% (v/v) pentanol-NaH_2PO_4 0.01 M (pH 6)-KCl 0.001 M, with electrochemical detection at 0.8 V.

RESULTS: The method had run time of 7 min, linearity greater than 0.999, limits of detection and quantification (ng/mL) of 4 and 12 respectively, and in and between-run precision CV's of < 1.8%.

CONCLUSIONS: The major advantage of the method is direct injection of biological and pharmaceutical or commercial samples by solubilizing the component in desired solvent. The addition of alcohols to the micellar phase results in an additional interaction with the solute. The variety of possible interactions gives a large versatility to this technique as an alternative to conventional HPLC and makes it appropriate for a wide range of solute analysis.

Extraction of Amphtamines and Methylenedioxyamphetamines in Urine Using Monolithic Silica Held in a Spin Column and HPLC-DAD Analysis

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AIMS: As a new tool for sample preparation of drugs in biological materials, monolithic silica was packed in a spin column. In this column, the structure of monolithic silica combined the support body and the surface area for each unit volume is wide in comparison to a particle-type silica. The handlings such as sample loading, washing, elution of target drugs, were exhibited by a centrifugation of the spin column. In addition, many samples can be processed at the same time. This method has many advantages; easy operation, low volume of extraction solvent, and without evaporation. In this study, the characteristics of the spin column hold by C_{18}-bonded monolithic silica were compared to those of a solid phase extraction cartridge. The pre-concentration efficiency of the spin column was excellent compared with the conventional solid phase extraction.

METHODS: Urine (0.5 mL), buffer (0.4 mL) and methoxyphenamine (IS) were put into the pre-activated spin column and the column was centrifuged at 3,000 rpm for 5 min. The column was then washed with the buffer by a centrifugation. Finally, the analytes adsorbed the column were eluted with the mobile phase (0. 2mL). 10 µL was injected on the HPLC.

RESULTS: The results demonstrated that the spin column was useful for extraction of amphetamines and

methylenedioxyamphetamines from urine. The higher pH buffer or lower pH buffer containing an ion pair reagent added to urine increased recovery, and better washing efficiency was obtained with the lower organic solvent concentration for washing. When 0.5 mL of urine was used for extraction, linearity was observed from 0.2 to 20 µg/mL with a correlation coefficients greater than 0.99. The CV's for intra- and inter-day variation at 1.0 and 10 ug/mL of amphetamines and methylenedioxy-amphetamines in urine were 1.8 and 10.7%. The minimum detectable levels in urine were 0.1 µg/mL.

CONCLUSIONS: Other samples were tested and good results were obtained. The sample preparation technique is being considered for use with other equipment, such as GC. This spin column has potential as a new tool for the routine extraction of drugs in biological materials.

Commercial SERS Substrates for a Roadside Screening Device for Drugs in Oral Fluids

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Drug driving and its detection is an ever increasing problem for police world wide. If a suitable roadside screening device for drugs (RSDD) was available then detection could become routine. In 2003 an amendment was made to section 6 of the UK Road Traffic Act 1988 which allows for collection of a specimen of sweat or saliva for use in a preliminary drug test. Collection of an oral fluid sample is seen to be the best approach for these roadside screening devices. This could feasibly be taken by an officer at the roadside when impairment was suspected, as additional confirmation to a positive field impairment test (FIT).

One possible approach to the development of an operational RSDD is the use of Raman spectroscopy. A Raman spectrum contains molecular fingerprint information that is specific to a particular drug. By placing the drug sample on a structured metal surface (SERS substrate) a greatly enhanced Raman signal is produced, this process is known as surface enhanced Raman spectroscopy (SERS). A device based around SERS could well provide the sensitivity, reliability and range of drug detection required for an effective RSDD. Commercial SERS substrates are now available, such as the Klarite substrate produced by Mesophotonics Ltd. The Klarite substrates feature a sub-micron scale patterning of a gold coated silicon surface. The surface is made up of a regular array of holes leaving a surface pattern that encourages the formation of surface plasmons, which govern the SERS amplification. If these commercially available substrates were to offer the sensitivity and reproducibility required for an RSDD then this could prove advantageous in the development of a working device.

A feasibility study has been undertaken to assess the suitability of the commercially available SERS substrates for a RSDD. Considerations include; sensitivity, reproducibility, and also sampling issues. The sampling issues include which laser wavelength is the most suitable to obtain maximum Raman scattering, and consideration of data variability within the sample spot.

Data was collected from five different concentrations of amfetamine sulfate in aqueous solution. Nine spectra were collected from three different positions within each sample spot; this was done for all five concentrations at two laser wavelengths, 633 nm and 785 nm. The data analysis for this work is currently underway and the results look promising. However, due to the high cost of these SERS substrates, the results of the feasibility study would have to be very favourable indeed for the slides to be cost effective for routine use at the roadside.

Experimental toxicology
Toxicologie expérimentale

Detection of Tramadol in Postmortem Rat Tissues

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AIMS: A clinical investigation on the development of dependence during oral therapy with tramadol for 153 hospitalized patients with daily dosages up to 400 mg over three was conducted. In order to evaluate the tramadol concentrations after chronic tramadol administration a rat model was used to predict the findings in human tissues.

METHODS: The LD_{50} (mg/Kg) of tramadol in mice and rats, respectively, is 200 and 286 subcutaneously, 350 and 228 orally, and 100 and 50 intravenously. Ten male albino rats were divided into two groups, each group consisting of five rats. The first group was given distilled water only and used as controls. The second group treated with tramadol at LD_{50} (52 mg/Kg) i.v. [1]. The rats were sacrificed after three house and dissected. Specimens collected included liver, kidney, brain, heart blood and hair. Tramadol was extracted from tissues and blood by liquid-liquid extraction using ammonium sulphate and methylene chloride [2]. Hair samples were washed in deionized water for five minutes to eliminate traces of blood. This was followed by three brief rinses in methanol to remove any other surface contaminations. Hair samples were subsequently dried and weighed. The hair was dissolved with sodium hydroxide, then hydrolysed with concentrated hydrochloric acid until a pH of 9 was obtained and extracted for tramadol by methylene chloride. Tramadol was quantitated by HPLC-UV using a C_{18} column, methanol : water (80:20) as a mobile phase (2 mL/min) with detection at λ = 254

nm. Chromatograms from control rats were used to compare HPLC chromatogram of treated rats. Tramadol metabolites were not evaluated in this study.

RESULTS: The distribution of tramadol in treated rats is summarized in the table below:

Distribution of tramadol (mg/Kg) in different organs after a dose of 52 mg/Kg.

Animal no.	Blood	Brain	Hair	Kidney	Liver
1	2,90	11,00	4,99	4,87	2,00
2	2,36	10,73	4,87	5,03	1,83
3	2,88	10,88	4,78	4,94	2,42
4	2,00	10,60	4,00	6,11	1,50
5	2,26	11,54	3,06	6,15	1,60
Average	2,48	10,95	4,34	5,42	1,67

CONCLUSIONS: From the present study we conclude that tramadol toxicity could be predicted in various tissues but it exhibits the highest concentration in brain, kidney and hair. The highest concentration of tramadol was detected in brain since the brain receives one-sixth of the total amount of blood leaving the heart. Lipid soluble drugs are distributed to brain tissue very rapidly compared with other tissues. Kidney has the next highest tramadol concentration since there is little tramadol excreted. Hair concentrations are next highest since tramadol moves by passive diffusion from the blood stream into the hair cells at the base of the follicle and are then bound in the interior of the hair shaft.

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Effects of Co-Administration of 3,4-Methylenedioxymethamphetamine, Methamphetamine, Ketamine and Caffeine on Plasma Concentration and on Urinary and Biliary Excretion in Rats

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AIMS: There has been a considerable increase in the number of seizures of amphetamine-type stimulant (ATS) tablets in Japan. The ATS tablets contain one or more active ingredients such as 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA). In addition, the tablets often contain other components with hallucinogenic and/or stimulant effects such as ketamine and caffeine that might interact in the body, leading to serious toxic symptoms. Therefore, the prediction of interactions among drugs is important for public health and forensic toxicology. We examined the interactions between MDMA, MA, ketamine and caffeine by co-administration of these drugs to rats as a model for potential pharmacokinetic interactions in humans taking ATS tablets containing various components.

METHODS: MDMA (10 mg/Kg) alone or with caffeine (50 mg/Kg) was administrated intravenously to Wistar rats (male, 200 - 250 g body weight). Similarly, MA (10 mg/Kg) alone or with ketamine (50 mg/Kg) was administrated. Blood was collected periodically from the tail vein (0.1 - 24 h) and urine was collected for up to 24 h. Bile was collected via a polyethylene tube inserted into the bile duct at determined time periods (up to 24 h). Plasma, urine and bile samples were hydrolyzed with 2 M HCl (120°C, 30 min) and deproteinized with acetonitrile. The samples were analyzed by LC-MS/MS for quantification of the drugs and their main metabolites under the selective reaction monitoring mode. Interactions between MDMA, MA, ketamine and caffeine were evaluated using pharmacokinetic parameters such as the area under the plasma concentration-time curve (AUC) and the amount of the drugs and their main metabolites excreted in urine and bile.

RESULTS: For plasma, the AUC of MDMA increased 2.1 times when co-administering caffeine compared to MDMA alone, while the AUC of MA decreased 0.45 times by co-administering ketamine compared to MA alone ($P < 0.05$, $n = 3$-5). The amount of MDMA excreted in urine up to 24 h was not changed by co-administering caffeine compared to MDMA alone, but the amount of MDMA excreted in bile was decreased (about 0.7 times) by co-administering caffeine compared to MDMA alone. On the other hand, the amount of MA excreted in urine and bile up to 24 h was not changed by co-administering ketamine compared to MA alone.

CONCLUSIONS: The AUC of MDMA significantly increased by co-administering caffeine. The inhibition of the biliary excretion of MDMA by caffeine might be a factor for the interaction between MDMA and caffeine. On the other hand, the AUC of MA significantly decreased by co-administering ketamine. The AUC decrease of MA may be due to interactions in the metabolism and/or distribution process. Similar interactions might occur in humans as well as rats.

Ethanol-Ecstasy (MDMA) Interactions in Rats: Effect on MDMA Pharmacokinetics and Body Temperature

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AIMS: Recreational use of Ecstasy, (±)-3,4-methylenedioxymethamphetamine (MDMA), is often associated with other drugs, among which ethanol is one of the most commonly used in dance clubs and rave cultures. We investigated the effects of ethanol co-administration on MDMA pharmacokinetics and body temperature in male Sprague-Dawley (SD) rats.

METHODS: Male SD rats (220 – 260 g) were obtained from CLEA Japan Inc. (Tokyo, Japan) and randomly divided into 6 groups (3 – 5 animals per group). The plasma concentration-time profiles were characterized after intraperitoneal (i.p.) administration of 10 mg/Kg and 30 mg/Kg MDMA alone and MDMA with 1.5 g/Kg ethanol to rats (4 animals per group). The rats were sacrificed 4 h after i.p. MDMA administration, and the plasma and brain samples were collected and analyzed using gas chromatography-mass spectrometry (GC-MS).

RESULTS: With 1.5 g/Kg ethanol, the maximum ethanol concentration in plasma was approximately 1.6 mg/mL after 0.3 h, and the average half-life was 2 h. Ethanol was not detected in the rat plasma after 4 h. However, ethanol concentration in the rat plasma did not statistically differ after the administration of MDMA in the presence or absence of ethanol. Following i.p. administration, the peak MDMA plasma concentrations (C_{max}) were obtained 0.189 – 0.275 h after the administration of both concentrations. The MDMA concentration in plasma significantly increased when co-administered with ethanol than when administered alone ($p < 0.05$). The pharmacokinetic parameters of MDMA in rats were adequately described by a 2-compartment open body model. Although N,α-dimethyl-(3-methoxy-4-hydroxybenzene) ethanamine (HMMA) and MDA are most abundant metabolites, we did not analyze HMMA. However, the (±)-3,4-methylenedioxyamphetamine (MDA) and MDMA concentrations in the brain did not differ among the 4 groups after 4 h. MDMA and MDA are well distributed in the brain when administered with ethanol. Although the temperature decreased after i.p. ethanol injection, it increased after the i.p. injection of MDMA.

CONCLUSIONS: After MDMA i.p. administration, plasma MDMA and MDA concentration will be increased and reach a higher value than p.o. administration. The enhancement of the effects of MDMA in the ethanol combination may be due to the initial increase in the MDMA plasma concentrations followed by the effect of MDMA and MDA in the brain.

Disposition of 4-Bromo-2,5-dimethoxy-phenethylamine (2C-B) and its Metabolite (4-Bromo-2-hydroxy-5-methoxyphenethylamine) in Rats after Subcutaneous Administration

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AIMS: 2C-B (4-bromo-2,5-dimethoxyphenethylamine) is a psychedelic abused drug inducing considerable euphoria in humans with an increased receptiveness of sensations. The pharmacokinetics of the agent in controlled studies in humans or animals is unknown. Due to ethical restrictions, our study was focused on assessing the distribution time profiles of 2C-B and its prevailing metabolite 2H5M-BPEA (4-bromo-2-hydroxy-5-methoxyphenethylamine) in serum, brain, liver and lung organs after a single drug dose to experimental rats with a focus on the brain/serum ratio.

METHODS: Male Wistars rats were subcutaneously administered a 50 mg/Kg bolus dose of 2C-B HCl in aqueous solution. The animals were sacrificed at 30, 60, 120, and 360 minutes after dosing (ten animals per time point) and the serum, brain, liver and lung samples were collected and stored at -20°C until analysis. The analytes were assayed by GC-MS as acetylderivatives.

RESULTS: The absorption of parent drug into blood stream was rapid; peak serum concentration was attained at 30 min after dosing (2250 ± 266 ng/mL) with a fast decline (estimated elimination half-time 1.1 h, distribution volume 16 l/Kg, clearance 9.8 l/h). 2C-B concentrations in all tissues peaked in 60 min and were higher than in serum (lung>brain>liver>serum). The highest concentrations were found in lung after 1 h (c_{max} 27028 ± 8156 ng/g) persisting high for up to 6 h, whereas the lowest concentrations were found in liver (c_{max} 7485 ± 1534 ng/g).The peak concentrations of the metabolite 2H5M-BPEA in tissues occurred within 1 h (liver, lung) or 2 h (brain) after the 2C-B dose. The concentration of 2H5M-BPEA metabolite in brain and lung were much lower relative to the parent compound reaching maxima of 3761 ± 1744 ng/g (lung) and 2726 ± 938 ng/g (brain). In liver, the principal organ for metabolism, the concentrations of 2C-B and its metabolite were relatively close. 2C-B distribution from blood into the brain was fast with an average peak concentration of 17102 ± 5202 ng/g (t_{max} = 1 h). The peak brain/serum ratio was 13.9 ± 1.9 at 2 h. The distribution of the hydroxylated metabolite into lipophilic brain tissue was less efficient relative to the parent drug.

CONCLUSIONS: The pharmacokinetic disposition of psychedelic 2C-B and its metabolite described above would be problematic to verify in humans. To our knowledge, our findings provide the first approximate estimation of kinetic data of 2C-B and its metabolite based on controlled animal experiments. The 2C-B temporal concentration profile in brain seems to correspond to 2C-B psychedelic temporal dynamic response reported. The drug's ability to accumulate in lung and persist in brain after a higher dose may explain the prolonged psychotropic effects. However, more experimental kinetic data are necessary, as there is a known steep dose response dependence.

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Influence of Drugs on Circadian Gene Expression in Cultured Rat Astrocytes

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AIMS: "There is none which is not a poison. The right dose differentiates a poison and a remedy (Paracelsus)" is a dogma in toxicology. Thus, the measurement of concentration of drugs in blood is important to evaluate the relationship between drugs and the cause of death. In some cases, however, although the drug concentrations are within therapeutic range, the cause of death seems related to drugs in the overall aspect. In such cases, physiological or pathological conditions, drug sensitivity, dose and drug combinations may play an important role in the development of toxic effects of drugs. For examples, polymorphisms of certain genes can be associated to impairment in metabolism of certain drugs. In this study, we focused on circadian rhythm as a general biological function that could affect drug toxicity.

METHODS: It is known that the efficacy and toxicity of drugs varies depending on the time of administration while little is known how drugs affect biological circadian systems. Victims who die of drug intoxication at relatively low concentrations are often habitual users of psychostimulants or psychotropic drugs. Recently, it has been clarified that circadian rhythms are generated and regulated by transcription/translation feedback systems of "circadian clock genes," such as period1, 2, 3, bmal1, and so on. We, therefore, investigated the effects of several drugs including dopamine on expressions of circadian genes using rat astrocyte cultures as a model system and evaluated changes of gene expressions by RT-PCR.

RESULTS: Dopamine induced rhythmic expression patterns of period1, 2, and bmal1 genes. Notably, acute elevation of period1 gene expression was prominent. The period1 expression increased by approximate 800% over the pre-treatment level 90 minutes after dopamine treatments (100 μM). The induction of period1 by dopamine was partially blocked with dopamine receptor antagonists. Methamphetamine and diazepam themselves did not increase period1 expression.

CONCLUSIONS: Although we do not have any data of in vivo experiments at present, our results suggest the possibility that some drugs that are involved in dopaminergic systems influence biological circadian rhythms.

Quantitative Analysis of mrt1b mRNA Expression Pattern in the Rat Brain Treated with PMEA

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AIMS: Sudden death following the use of PMEA (p-methoxy-ethylamphetamine) is a growing problem in Japan, however its pharmacological cause remains to be elucidated. The present study was conducted to obtain clues to the cause of sudden death for clarifying a mechanism of PMEA. Repeated administration of methamphetamine (MA) produces an enduring hypersensitivity to MA, termed behavioral sensitization. First of all, we examined the expression pattern of a novel stimulant-inducible gene mrt1b (methamphetamine responsive transcript 1b) encoding a PDZ-PX protein in stimulant-induced behavioral sensitization. Subsequently, we investigated whether PMEA could upregulate the expresssion of mrt1b transcripts.

METHODS: In the young adult rats, repeated daily treatment with MAP (2.0 mg/Kg, IP, once a day) or PMEA (10 mg, 20 mg, 30 mg/Kg, IP, once a day) for 14 days induced an enhanced behavioral sensitization. After a 7-day withdrawal period, rats were sacrificed. The cerebral cortex was rapidly removed in the cold and stored. Total RNA was extracted using a total RNA isolation kit and cDNA was synthesized by using a first strand cDNA synthesis kit. Expression pattern of mrt1b mRNA was measured by a quantitative RT-PCR method.

RESULTS AND CONCLUSIONS: The 14-day administration of MA upregulated the basal expression of mrt1b mRNA. In contrast, rats treated with PMEA failed to elicit a significant change in the mrt1b mRNA expression when compared to controls. Moreover no changes in basal expression of mrt1b mRNA were

observed with a lower dose of PMEA (1 mg, 3 mg, 5 mg/Kg). These findings raise the possibility of different mechanisms of the regulation of mrt1b expression, suggesting that PMEA would not contribute to behavioral sensitization.

Behavioral Effects and the Time Profile of the Psychedelic 2C-B in Blood and Brain Tissue of Experimental Rats

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AIMS: Nexus, 4-bromo-2,5-dimethoxyphenethylamine (2C-B) is one of the synthetic psychedelics, which can modify subject's perception and psychomotor functions. 2C-B is known to produce euphoria and sensory enhancements with effects in humans lasting up to 8 hours. The steep dose-response relationship has been reported. To date, no controlled pharmacokinetic study related to behavioral observation has been performed. The aim of our study was to evaluate behavioral effects with respect to the disposition of 2C-B in brain after subcutaneous administration to experimental rats.

METHODS: All experiments were carried out on male Wistar rats (200-250 g). In behavioral experiments we have focused on locomotor effects of the drug and on changes in sensorimotor gating. We used four increasing doses of 2C-B (2.5, 10, 25 and 50 mg/Kg). The drug was dissolved in physiological saline and injected subcutaneously 15 minutes before measurements. Locomotor activity was registered for 30 minutes via an automatic video tracking system for recording behavioral activities (EthoVision Color Pro v. 3.1.1, Noldus, Netherlands). Sensorimotor gating was measured as prepulse inhibition of acoustic startle reaction (PPI). The testing was performed in the startle chamber (SRLAB, San Diego Instruments, California, USA). In the pharmacokinetic study, the animals were injected single subcutaneous bolus dose 2C-B hydrochloride salt 50 mg/kg in physiological saline and rats were sacrificed after 30, 60, 120, and 360 min (ten animals per time). After sampling, blood sera were separated and sera and whole brains were kept frozen at –20°C till analyses. 2C-B was isolated from sera and brain homogenates after addition of internal standard MBDB using solid phase extraction discs (SPEC-DAU). After acetylation, the analysis was performed by GC-MS in SIM mode. All experiments respected the Guidelines of the European Union Council (86/609/EU) and followed the instructions of the National Committee for the Care and Use of Laboratory Animals.

RESULTS: In locomotor experiments 2C-B significantly decreased locomotion of animals. This effect was apparent mainly during the first 10 minutes of testing. In the test of PPI of acoustic startle the drug significantly disrupted prepulse inhibition and 2C-B also significantly decreased the startle reaction. After the high drug dose (50 mg/Kg), the maximum serum concentration was attained 30 min after the administration with the mean c_{max} 2250 ng/mL and subsequent fast decline with estimated half time 1.1 h. The maximum concentration in the brain was attained after 60 min with mean maximum value c_{max} 17,102 ng/g. The maximum brain to serum ratio (mean value 13.9) was attained after 120 min. The brain to serum ratio remained high still at 360 min and it indicates prolonged psychedelic effects after higher doses.

CONCLUSIONS: We may assume that 2C-B is behaviorally active in rats. It has an inhibitory influence on locomotor activity and disrupts sensorimotor gating. This effect is comparable to other hallucinogens. Behavioral effects were pronounced even though the drug has not reached a maximal brain concentration yet, indicating that these can be even more expressed later.

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Detection Time of Phentermine in Blood, Hair and Urine of Rats

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AIMS: The aims of this study are to determine the detection time of phentermine in blood, hair and urine samples and to establish if any correlation exits between drug dose and detection time using a rat model.

METHODS: Four groups of three male albino rats of approximately the same weight and age were given phentermine, IP, in doses 5, 10, and 15 mg/Kg of the body weight designated as Group A, B and C respectively. Group D served as a control. Samples were collected as available (blood: 0.5, 2, 4, 8, 12, 24 and 48 h; urine: 2, 6, 16, 24, 36, 64, 89, 120, and 148 h; hair: 7, 14, 21, 28, 35, and 42 days). After appropriate sample pre-treatment (blood: protein precipitation; hair: alkaline hydrolysis) phentermine was isolated using a Toxi-Tube A extraction system. Phentermine was quantitated by HPLC-UV using established procedures (Manual for Use by National Laboratories. United Nations. New York. 1997).

RESULTS: Phentermine could be detected in blood in all samples from 0.5 - 12 h and up to 24 h except for the lowest dose. In urine, phentermine was detected beginning at 6 h (except 16 h for the lowest dose) through 120 h and up to 148 h for the highest dose. For the lowest dose, phentermine was only detected in hair on

day 21. However, for the highest dose, phentermine was detected in all specimens collected. For the intermediate dose, phentermine was detected from day 14 to day 35. Peak concentrations occurred 4 h, 64 h and 21 days for blood, urine and hair, respectively (Table 1).

Table 1: Average Peak Concentrations of Phentermine (P) at Different Doses Administered

Dose (mg/Kg)	Blood		Urine		Hair	
	P Conc (ppm)	% P retained	P Conc (ppm)	% P retained	P Conc (mg/mg)	% P retained
5	2.24	44.8	1.60	32.0	0.71	14.2
10	4.32	43.2	3.10	31.0	1.08	10.8
15	8.14	54.3	4.65	31.0	1.25	8.3

The results showed that it took the longest period of time for phentermine to be detected in hair, followed by urine and blood. However, the concentration of phentermine detected was highest in blood followed by urine and hair. Generally, there was an increase in the concentration of phentermine in all matrices as the dose administered increased.

CONCLUSIONS: This study demonstrates that phentermine concentrations and detection times in blood, urine and hair of rats are dose-dependant. Negative results in some samples may be due to either the absence of phentermine or due to concentrations below the detection limit of the assay.

A New Concept for Oral Fluid Sampling, Storage and Recovery

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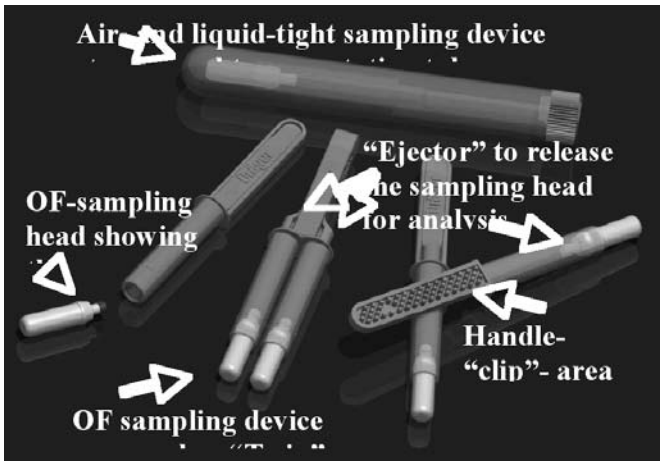
AIMS: As an ultra-filtrate of the blood, saliva (oral fluid) is scientifically recognized and well-established in clinical chemical diagnostics. Oral fluid samples are widely accepted as specimens for on-site and lab-based drug of abuse diagnostic analysis. Reliable confirmation of screening results and an accurate lab-based analysis both require a sample collection system that does not adversely affect the level and nature of analytes contained in the specimen during storage, transportation and recovery. The work presented describes a new oral fluid sampling, storage and transportation concept – the "Dräger DCD 5000™".

METHODS: The sampling-head of the device – a non-compressible porous body - indicates visually sample adequacy after the sampling process and virtually excludes intended or unintended manipulation and falsification of the sample. For the validation studies, up to 30 of this new oral fluid collection devices were loaded with pooled oral fluid, spiked with drugs of abuse (e.g. 23 ng/mL Δ^9-THC, 20 ng/mL morphine).
The devices were stored up to 16 days at 21°C; recovery was determined by GCMS analysis.

RESULTS AND CONCLUSIONS: The GCMS-results revealed as provisional result nearly 90% recovery of the original spiked analyte amount after 10 days storage (result up to 16 days in progress). Reliable drug recovery rates and defined losses due to e.g. adsorption to the storage and transportation tube or collection device housing/handle will be achieved. The device can be used to collect oral fluid samples quickly, efficiently and without pain. Due to the minimally invasive sampling process and easy handling during operation and for recovery (e.g. ejector disposition), the risk of infection for medical or lab personnel is substantially reduced. The concept is also suitable for single or "twin" sample collection, offering a practical undiluted, non-chemically-treated sampling split approach.



Analysis of Morphine in Hair by HPLC-Fluorescence Detection after Administration in Rats

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AIMS: The determination of morphine (Mor) in hair is important as Mor is not only a drug of abuse but an analgesic drug used for both post-operative and cancer pain. Therefore, an HPLC-fluorescence detection method for Mor in hair was developed. Sensitive determination was achieved by labeling with 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride (DIB-Cl). The proposed method was applied for the determination of Mor in hair after administration to rats.

METHODS: Mor (10 mg/Kg, i.p.) was administered to Zucker male rats once a day for 5 days. Black and

white hair samples were obtained from the same part by shaving at 7, 14 and 21 days after the last administration. The hair, including the root, was obtained by tweezing at 30 day. The hair sample was washed with 0.1% SDS solution and distilled water and extracted with 5% TFA in MeOH (1 mg hair/mL). A 100 µL portion of extract was taken and dried with N2 gas. To the residue, 0.4 M carbonate buffer (pH 10) and 5 mM DIB-Cl suspension in acetonitrile were added. The mixture was allowed to stand at room temperature for 10 min and then the reaction was stopped by adding 10 µL of 28% NH_3. The sample was extracted using solid-phase extraction (BondElut®C18) and analyzed by HPLC. The sepa–ration of DIB-Mor was achieved by an ODS column (250 x 4.6 mm) with 0.1 M acetate buffer : CH_3CN (50:50, v/v) and monitored at 355 nm (Ex) and 486 nm (Em). The HPLC analysis of DIB-Mor including column washing with acetonitrile was completed within 33 min.

RESULTS: The calibration curve for Mor (0.25 to 20 ng/mg) showed a good linearity (r = 0.999). The detection limit of Mor at a signal-noise-ratio of 3 was 82 pg/mg. The intra- and inter-day precision studies demonstrated CV's of less than 6.0%. The quantitation of Mor in hair after i.p. administration was performed. Mor could be detected in the black hair the 7th day after administration (8.02 ng/mg) including the root (0.47 ng/mg). Mor could not be detected in any white hair samples.

CONCLUSIONS: The proposed method was sufficiently sensitive and precise for the determination of Mor in hair samples. Therefore this method might contribute the clinical, forensic and toxicological studies of Mor.

Analysis of 10 Anabolic Steroids in Hair by High Performance Liquid Chromatography Electrospray Tandem Mass Spectrometry

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AIMS: To establish a sensitive and reproducible method for detection, quantification and confirmation of ten major anabolic steroids (AAS) (methandienone, boldenone, nandrolone, oxymesterone, testosterone, epitestosterone, methyltestosterone, methenolone, norethandrolone, stanozolol) in hair by liquid chromatography electrospray tandem mass spectrometry.

METHODS: After decontamination steps, the hair sample (about 50 mg) was solubilised in 1 mL 1M NaOH, 2 h at 80°C, in presence of 1 ng of epitestosterone-d_3 used as internal standard. The homogenate was neutralized and extracted by liquid–liquid extraction using pentane. The extract was separated by reverse phase liquid chromatography using methanol-water (20 mmol/L ammonium acetate) as mobile phase. The AAS was confirmed and quantified using an API4000 MS-MS system in the multiple reaction monitoring (MRM) mode via positive electrospray ionization. Stability of AAS in NaOH at 80°C was evaluated over 3 h by comparing peak area of analytes spiked in 1 M NaOH with those spiked in water. Any matrix effect was evaluated by comparing peak area of analytes in blank hair samples spiked after the sample preparation with those obtained by direct injection of chemical standards. Stability and matrix effects were both evaluated at three (low, medium, and high) concentrations (n = 5 each).

After a single intraperitoneal injection of 60 mg/Kg stanozolol and methyltestosterone to 4 guinea pigs, respectively, hair samples were collected every other day from days 2 - 14 after administration for the measurement stanozolol and methyltestosterone in hair.

RESULTS: The AAS in 1 M NaOH incubated at 80°C for 3 h showed a less than 5% degradation. The calibration curves for the AAS demonstrated excellent correlation coefficients (r^2 = 0.9958 - 0.9998). Precision studies ranged from 1.7 - 13.8% RSD and recoveries were between 38.2% and 110.4%. The limits of detection from 1 to 20 pg/mg. The method showed little or no matrix effect from hair (i.e. stanozolol 93.8 - 107.8%). The analysis of hair obtained from guinea pigs revealed the presence of stanozolol (10.0 to 506.0 pg/mg) and methyltestosterone (10.0 to 641.0 pg/mg).

CONCLUSIONS: The established method is simple, sensitive and specific and was successfully applied to the analysis of stanozolol and methyltestosterone incorporated into guinea pig hair.

Methamphetamine Incidence and Concentration Found in Hair Samples

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AIMS: Hair analysis is commonly used to identify long and continuous use of drugs of abuse such as cannabis (THC), cocaine (COC), amphetamines (AMPS), opiates (OPI) and phencyclidine (PCP). We evaluated the results of 260 samples that were collected in our laboratory and analyzed for amphetamines.

METHODS: Hair samples, which were collected from the crown area of the head, were submitted for analysis to one of two laboratories, which we use for our analyses. In each case, samples were screened by immunoassay and confirmed by gas chromatography mass spectrometry (GC-MS or GC-MS/MS). Cut-offs used to differentiate amphetamine positives from negatives, were 300 pg/mg for screening and 300 pg/mg for confirmation.

RESULTS: From a population of 260 samples tested, 129 were females (50%), 120 were males (46%) and 11 (4%) were not identified by sex. Of the 260 samples tested, 73 or 28% were positive for drugs. Of these, 29 or 40% were positive for amphetamines. For the 29 positive amphetamine samples, 16 or 55% were female and 13 or 45% were male. The concentration range of amphetamines was as follows: amphetamine (AMP) 304 to 5632 pg/mg, methamphetamine (METH) 455 to 42746 pg/mg. The mean AMP and MAMP concentrations were 1628 pg/mg and 7278 pg/mg, respectively. The mean ratio of metabolite to parent drug (AMP / METH) was 12%. A plot of methamphetamine against amphetamine concentration showed a linear relationship with a slope of 0.0983, intercept of 235.34 and correlation coefficient r of 0.77 ($r^2 = 0.60$).

CONCLUSIONS: The incidence of amphetamines in 40% in this population appears to be high. The incidence of amphetamine use appears to be slightly lower for males than for females. What is most alarming is that methamphetamine use appears to be the number one abused drug in this population group.

Use of Hair Analysis for Medico-legal Purposes Exemplified in a Fatal Case Involving a Profile of Opiate and Cocaine Abuse

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AIMS: The subject of the study presented in this work is a fatal case of a female who was an opiate and cocaine abuser. The toxicological analysis of the blood of the victim was carried out to determine the cause of death and segmental hair analysis was performed as a means to evaluate a multi-parameter drug abuse profile retrospectively.

METHODS: The venous autopsy blood and hair of the victim were subjected to solid phase extraction (SPE) and analyzed with the use of liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS-MS). The limit of quantitation (LOQ) for cocaine, benzoylecgonine, morphine, codeine, 6-MAM in blood was 1.0 ng/mL. The LOQ in hair analysis for cocaine and benzoylecgonine was 0.05 ng/mg; for morphine, codeine and 6-MAM the LOQ was 0.2 ng/mg. Linearity was obtained up to 2000 ng/mL and 50 ng/mg for cocaine and opiates in blood and hair respectively. Intra-assay accuracy and precision ranged from 1.2 to 12.2 and 1.4 to 4.7%, respectively. Inter-assay accuracy and precision ranged from 1.4 to 12.2 and 3.1 to 7.3%, respectively.

RESULTS:

Xenobiotics	Concentration in blood (ng/mL)	Concentration in hair (ng/mg)			
		S_1	S_2	S_3	S_4
Cocaine	38.1	29.1	29.1	34.6	47.2
Benzoylecgonine	1840	8.0	5.7	5.2	6.7
6-MAM	-	0.7	0.7	1.1	1.8
Morphine	72.3	-	-	-	-
Codeine	30.2	-	-	-	-

(S_n) - number of segment hair, length of each was 2 cm ; (-) - not detected

CONCLUSIONS: Morphine, codeine, cocaine and benzoylecgonine were detected in blood at concentrations resulting in the cause of death of the woman being explained as cocaine-opiate abuse. Segmental hair analysis confirmed the presence of a cocaine-opiate abuse profile for at least 8 months before death.

Development and Validation of a Method for the Quantitation of Oxazepam and Oxazepam-Glucuronide in Serum, Urine and Oral Fluid

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AIMS: To develop and validate an analytical method that is suited for the determination of oxazepam and its major metabolite, oxazepam-glucuronide, in serum, urine and oral fluid.

METHODS: Extracts of serum, urine and oral fluid were analyzed by using HPLC-MS/MS. The analytical system consisted of an Acquity® UPLC, using a C18 column and a methanol/formic acid gradient, coupled to a Waters Quattro Premier® tandem MS. Several methods of extraction were tested. For serum, these were: liquid/liquid extraction (on ChemElut® matrix), SPE with Oasis® HLB (1 cc and 3 cc columns), SPE with Extract-clean® DVB and protein precipitation with various solvents. For oral fluid, SPE with Oasis® HLB (3 cc) and protein precipitation were compared. Extracts were dried (75 min at 45°C in vacuum) prior to analysis. The run time of the HPLC method was chosen so as to combine a short run time with minimal ion suppression. Stability was tested during a period of 4 months at -18°C.

RESULTS: SPE extraction with a single elution step gave unsatisfactory results for all matrices, especially for oxazepam-glucuronide. Protein precipitation with either methanol, acetonitrile, DMSO/phosphoric acid or acetone gave acceptable results for both oxazepam and oxazepam-glucuronide. Drying of the extracts at 45°C

did not lead to degradation of the compounds.
The final method consists of protein precipitation with methanol, followed by centrifugation and HPLC-MS/MS analysis. D5-oxazepam and lorazepam-glucuronide are used as internal standards. The run time is 5 minutes. Validation data: Linearity 0.5 - 100 ng/mL (oral fluid); 1 - 1000 ng/mL (serum, urine), for oxazepam and oxazepam glucuronide. Limit of detection 0.25 (oral fluid); 0.5 (serum) and 0.5 (urine) ng/mL for oxazepam and 0.25 (oral fluid); 0.5 (serum) and 0.5 (urine) ng/mL for oxazepam glucuronide. Limit of quantification 0.5 (oral fluid); 1 (serum) and 1 (urine) ng/mL for oxazepam and 0.25 (oral fluid); 1 (serum) and 5 (urine) ng/mL for oxazepam glucuronide. Extraction recovery: 70 - 105% for oxazepam and oxazepam glucuronide, depending on the matrix. Intra-day precision was less than 15 - 20%. Inter-day precision was not determined because calibration curves were included in each analytical run. No degradation was observed after 4 months storage at -18°C in serum and oral fluid, however in urine the oxazepam-glucuronide concentration decreased by 24% in this period.

CONCLUSIONS: For oxazepam and oxazepam-glucuronide, compounds with strongly differing polarities, protein precipitation was the extraction method of choice. Based on protein precipitation with methanol, a quantitative method for the analysis of oxazepam and oxazepam-glucuronide in serum, urine and oral fluid in the sub-ng/mL range was developed and validated.

The Determination of Ketamine Concentrations in Hair Samples

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AIMS: The aim of this poster is to provide information on the ketamine levels detected and the prevalence of the drug use by using hair samples collected in the UK. Since 2002, TrichoTech has received 2,954 samples of hair requiring screening for ketamine by enzyme linked immunosorbent assay. 179 of these samples were analysed by electron impact - gas chromatography mass spectrometry (EI-GCMS).

METHODS: The hair sections analysed were weighed and then washed with a solvent. The hair samples were submitted to alkaline digestion and then liquid : liquid extraction with a mixture of chloroform : isopropanol. After solvent evaporation the residues were reconstituted in phosphate buffer, pH 7.2. For the confirmation analysis by EI-GCMS, the samples were extracted using OASIS MCX solid phase extraction cartridges (Waters, Elstree, UK) or HCX Solid Phase Extraction cartridges (IST, Hengoed, UK). Prior to 2006 samples were analysed underivatised; since 2006 samples have been derivatised with trifluoroacetic acid anhydride (TFAA) (Sigma, Poole, UK) to produce the trifluoroacetyl (TFA) derivative. The samples were analysed by EI-GCMS using HP5973 (Agilent, Wokingham, UK) using single ion monitoring. The ions scanned for the d4-ketamine were m/z 274 and 302, and the ions scanned for ketamine were m/z 270, 236 and 298. Ketamine and d4-ketamine reference standards were acquired from Cerilliant (LGC Promochem, Teddington, UK). The uncertainty of measurement (UM), which is the associated variability of the analytical tests, was calculated by multiplying the relative standard deviation by 2 to provide a level of confidence of approximately 95%.

RESULTS: The calibration curve for ketamine was measured over the range 3.6 to 120 ng/mL. The uncertainty of measurement for ketamine was 6% calculated from intra- and inter-day quality controls. The cut-off and limit of quantitation for the assay was 0.2 ng/mg hair (assuming a 10 mg sample). Ketamine was detected in 65 hair sections. The results are shown in Table 1.Table 1. Ketamine Concentrations in Hair – Grouped by Levels Detected.

Ketamine concentrations (ng/mg hair)	No. Samples
0-10	24
10-50	17
50-100	10
100-200	6
> 200	8
Total	65

CONCLUSIONS: The validated method is both sensitive and reproducible for the quantification of ketamine in hair samples. The uncertainty of measurement of ketamine was determined by the EI-GCMS method and was found to be 6%. The concentrations detected in the 65 samples confirmed by EI-GCMS ranged from 0.5 to 2818.5 ng/mg of hair. The highest frequency of results was in the 0.2 to 10 ng/mg hair range. The median was 21.4 ng/mg hair. The percentage of hair samples submitted to the laboratory for ketamine analysis with the presence of ketamine detected by EI-GCMS was 2.2%. Where ketamine was detected, 75.4% of the hair samples also tested positive for at least one other drug tested concomitantly at TrichoTech. The most common drug used was cannabis (60%), followed by cocaine (38.5%) and then amphetamine and methamphetamines (36.9%)

The Evaluation of Amphetamine Isomer Ratios in Hair Samples from Amphetamine Users

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AIMS: The aim of the study was to examine the pattern of L/D amphetamine ratios in relation to the amphetamine concentrations detected in hair samples

and the declaration of amphetamine based medication. Amphetamine, a stimulant drug, is available on prescription in many countries for the treatment of several disorders such as narcolepsy or obesity. In the UK amphetamine is available as Dexedrine (dexamphetamine) and used in the treatment of drug abuse. It is also used illegally for recreational purposes and is currently a Class B controlled drug or class A if prepared for injection. Amphetamine is a chiral compound consisting of L- and D- isomers (also known as R-(-)/S-(+)). The L/D ratio for Dexedrine is around 5% whilst that of illicit amphetamine is 100% (1).

METHODS: The determination of the enantiomeric composition of amphetamine in urine and blood samples has been used as a valuable tool in interpreting drug-testing results for the management of amphetamine users (1). Samples were extracted using a SPE cleanup followed by derivatisation using N, O-bis(trimethylsilyl) trifluoroacetamide. Samples were injected onto a Varian 2000 GC-MS/MS, using a GC column. (Restek, Rt-BDEXcst-TM; 30 meter, 0.25 mm ID, 0.25 µm df). The benefits of the assessment of amphetamine enantiomer distribution using hair analysis has been highlighted previously but on small group of patients (2,3). This study examines the pattern of L/D amphetamine ratios in relation to the amphetamine concentrations detected in 106 hair samples obtained from drug users who had either self declared or not declared amphetamine use.

RESULTS: The overall pattern of results suggests that the population that have declared drug use are less likely to be supplementing with 'street' amphetamine where high amphetamine concentrations have been found. Whereas, the population where drug use has not been declared are more likely to be supplementing with 'street' amphetamine, also, where concentrations of amphetamine have been found.

Dexe-drine	Interpretation	N	L/D Ratio		Amphetamine	
			Mean	(SD)	Mean	(SD)
Declared	Prescribe Dexedrine only (ratio <=20%)	27	5,6	(4,0)	17,1	(26,2)
	Supplementing with illicit amphetamine (ratio 20 - 50%)	9	30,2	(8,2)	45,7	(43,6)
	Use mainly illicit amphetamine (ratio >50%)	5	84,0	(20,7)	89,4	(113,0)

CONCLUSIONS: The results confirm previous data that hair analysis can be employed as a useful technique in monitoring amphetamine compliance over a long period of time.

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An Evaluation of a Point-of-Contact Test for (Δ^9-THC) in Oral Fluid

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AIMS: Oral fluid collection is non-invasive, quick and easy to perform, and testing detects primarily the parent drug and lipophilic metabolites. The Cozart® DDS system combines rapid and adequate collection of oral fluid (indicated by the sample presence indicator) with reliable recovery of drugs from the collection media, a rapid point-of-contact (POC) test cartridge and an instrument which interprets the results. We report here an evaluation of the Cozart® DDS system in terms of its sensitivity and specificity to Δ^9-THC in samples collected from drug users attending drug rehabilitation clinics.

METHODS: Oral fluid samples were collected using the Cozart® DDS oral fluid collector. The device collects an average volume of 0.34 mL (Standard deviation = ± 0.06) of oral fluid in just under 1 minute. Samples were collected from drug users attending a number of drug rehabilitation clinics across the South of England and Wales. All samples were automatically diluted 1 in 3 with the Cozart® DDS Buffer as detailed in the collection protocol. The Cozart® DDS 5 drug panel test for opiates, cocaine, methamphetamine (ecstasy), amphetamines and cannabis (Δ^9-THC) was conducted on site, at the time of collection, and the remainder of the sample retained for confirmation analysis. The standard cartridge run time of 5 minutes was used. The results of the point-of-care test were obtained using the Cozartâ DDS reader which displayed the results on the screen and provided a printed copy. The concentration of Δ^9-THC in the oral fluid was determined by tandem MS (L.O.D. 1 ng/mL, LOQ 1.5 ng/mL) and then compared to the result of the on-site test to establish the sensitivity and specificity.

RESULTS: A total of 20 control (drug-free) samples and 44 clinic samples were tested. All the drug-free samples were negative for cannabis when tested by the DDS device and confirmed by tandem MS. 22 out of the 44 clinical samples were cannabis positive by the DDS of which, 21 were confirmed positive by tandem MS. The concentration of Δ^9-THC ranged from 5.4 ng/mL up to a maximum of 4218ng/mL with an average of 507 ng/mL. The comparison between confirmed results and the POC test results showed that of the 64 samples tested there was 1 false positive and 1 false negative. Therefore the accuracy of the Cozart® DDS test was 97%, the sensitivity was 95.5% and specificity was 97.6%. The cross-reactivity of various compounds, at 100,000 ng/mL, were tested and found not to cause a false positive result for Δ^9-THC on the Cozart® DDS 5 drug cartridge.

CONCLUSIONS: Oral fluid is widely accepted as a matrix that identifies recent drug use and related impairment. Our results show that for the identification

of Δ^9-THC in oral fluid samples, the Cozart® DDS System is an accurate screening tool which can be easily used at the point of care. The instrument provides an accurate interpretation of the result and removes operator subjectivity or bias. In conclusion, the Cozart® DDS System provides an on-site sensitive, specific and reliable test for cannabis (Δ^9-THC).

Automated Immunoassays for the Detection of Opiates, Amphetamines and Methamphetamines in Oral Fluid on Roche Instrument Platforms

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AIMS: Assays are in development for the detection of opiates, amphetamines, and methamphetamines in oral fluids on automated clinical analyzers**. The liquid, homogeneous assays utilize the KIMS technology (Kinetic Interaction of Microparticles in Solution), where a monoclonal antibody is covalently linked to carboxy-modified polystyrene microparticles, and a drug conjugate is in solution. We present here the adaptation of automated immunoassays for the detection of opiates, amphetamines and methamphetamines in oral fluid on Roche instrument platforms.

METHODS AND RESULTS: The Opiates oral fluid assay utilizes a cutoff concentration of 10 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff is equivalent to approximately 40 ng/mL in undiluted oral fluid, per the proposed SAMHSA guidelines. When run in a semi-quantitative mode based on a six-point calibration on a Roche/Hitachi 917 analyzer, control recovery for samples at ±25% of the cutoff showed a recovery of 7.7 ng/mL (103%) and 12.5 ng/mL (100%) with %CV precision values of 2.8% and 1.5%, respectively. Between patient variability of samples spiked at concentrations of 8.0, 10.0, and 12.0 ng/mL morphine ranged from 94 - 105% recovery. The Amphetamines and Methamphetamines assays utilize cutoff concentrations of 12.5 ng/mL when using the Intercept® Oral Specimen Collection Device. For each of the assays, the cutoff is equivalent to 50 ng/mL in undiluted oral fluid. The amphetamine and methamphetamine assays are separate reagent systems that use d-amphetamine and methamphetamine calibrators, respectively. Control recovery for samples ±25% of the cutoff on the amphetamines assay showed a recovery of 9.6 ng/mL (102%) and 16.0 ng/mL (103%) with %CV precision values of 4.8% and 1.4%, respectively. Control recovery for samples ±25% of the cutoff on the methamphetamines assay showed a recovery of 9.5 ng/mL (101%) and 16.2 ng/mL (104%) with %CV precision values of 6.2% and 5.2%, respectively. The methamphetamines assay has a cross-reactivity of 100% to MDMA and 75% to MDEA.

CONCLUSIONS: The opiates, amphetamines and methamphetamines assays offer accurate and precise methods for the quantitation of these drugs of abuse in oral fluid samples on automated systems.

***These assays are currently in development and have not been approved for use in the US by the FDA.*

Oral Fluid Collection Devices: Recovery and Stability of Drugs

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The aim of this study was to investigate the recovery and stability of selected drugs in oral fluid (OF) collected with nine different collection devices. The investigated devices were Greiner Bio-One, Orasure Intercept®, Immunalysis Quantisal™, Statsure Saliva Sampler™, Cozart®, Sarstedt Salivette®, Malvern Medical OraCol, Acro Biotech Salicule, Varian OraTube™. For comparison, OF was also collected into plastic tubes (Sarstedt). 1 mL of OF spiked with different drugs (amphetamine, MDMA, cocaine, Δ^9-THC, morphine, codeine, diazepam and alprazolam, all 1000 ng/mL in OF) was added to each device and stored according to the instructions of the manufacturer. The recovery was calculated for each substance from six replicates. For stability studies, six replicate samples were analysed right after preparation. Another six samples were analysed after 14 days and 28 days of storage.

For analysis, a calibration curve was prepared in OF. The samples were extracted with ethyl acetate (including deuterated analogues as internal standards for all analytes) at pH 10. The solvent was then separated and evaporated. The residue was derivatised with ACN-MSTFA. The samples were analysed with GC–MS.

Recovery range for all analytes excluding Δ^9-THC was:

Percentage	Device	Percentage	Device
86.4% - 98.5%	Greiner Bio-One	15.9% - 51.8%	Sarstedt Salivette®
88.9% - 116%	Orasure Intercept®	<10% - 69.8%	Malvern Medical OraCol
81.1% - 111%	Immunalysis Quantisal™	92.2% - 99.6%	Acro Biotech Salicule
81.3% - 91.1%	Statsure Saliva Sampler™	39.8% - 86.7%	Varian OraTube™
66.0% - 91.6%	Cozart®		

For comparison, the same range for ordinary plastic tube was 93.6% - 102%.

For Δ^9-THC, recoveries were:

Percentage	Device	Percentage	Device
73,6%	Greiner Bio-One	<10%	Sarstedt Salivette®
37,6%	Orasure Intercept®	<10%	Malvern Medical OraCol
55,8%	Immunalysis Quantisal™	45,9%	Acro Biotech Salicule
85,4%	Statsure Saliva Sampler™	47,5%	Varian OraTube™
75,9%	Cozart®	74,6%	plastic tube

The stability of the analytes was fairly good for all devices (only descent of 10 - 30 percentage units in analyte concentrations during 28 days storage for all the devices). However, it is essential to note that the overall recovery of the analytes after the whole sampling process is of primary importance when selecting an OF collection device for toxicological analysis. As a conclusion, considerable differences in the overall reliability of OF collection devices were noted. The results of the study emphasize the impact of the selection of the OF collection device on the whole toxicological procedure.

Homogeneity Study in the Preparation of a Reference Material Using Methamphetamine Abusers' Hair Samples

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AIMS: The need for certified reference materials for drug analysis has rapidly increased in forensic and clinical laboratories as part of quality control. This study presents the results of a homogeneity testing for the preparation and certification of a reference material (RM) for the determination of methamphetamine (MAMP) and its main metabolite, amphetamine (AMP), in human hair using authentic hair samples.

METHODS: Methamphetamine abusers' hair samples, where the MAMP concentrations ranged from 0.5 to 50 ng/mg, were washed in dichloromethane for 2 min twice, dried, cut into less than 2 cm and stirred for 60 min in distilled water. After drying again, the hair was segmented into about 1 mm, sieved, blended and finally bottled (103 vials, ca. 100 mg each). In order to evaluate the homogeneity among the bottles, 30 samples were taken out of a batch and the concentration of each sample was determined using two extraction methods, one based upon agitation with 1% HCl in methanol at 38°C and one based upon ultrasonication with methanol/5M HCl (20:1). Both methods were followed by gas chromatography/mass spectrometry (GC/MS) after derivatization with trifluoroacetic anhydride (TFAA) and uncertainties were calculated.

RESULTS: The results from the two methods were in good agreement with the concentrations of 7.8 and 7.7 ng/mg for MAMP as well as 0.53 and 0.55 ng/mg for AMP. The uncertainties were 3.9% and 2.3% for MAMP as well as 0.72% and 2.8% for AMP.

CONCLUSIONS: Satisfying homogeneity was reached for MAMP and AMP in the prepared RM. This specimen can be provided gladly to any laboratories for internal quality control and research purposes.

Drug Use Pattern in Hair and Blood in Deceased Drug Addicts

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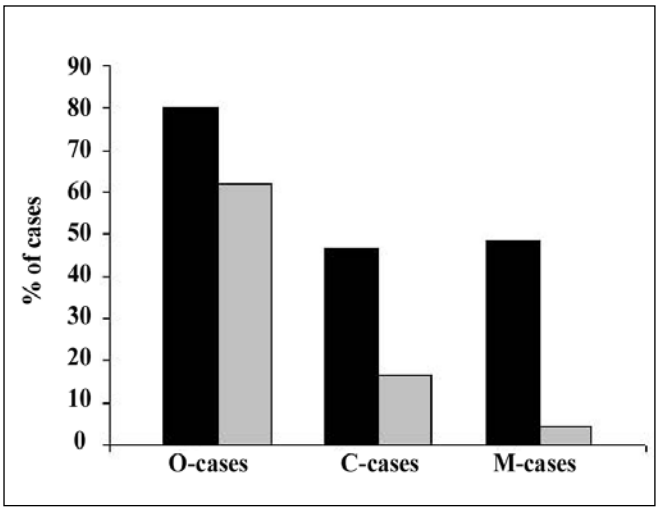
AIMS: The risk factors associated with opiate overdose death call for detailed characterization. In the absence of a biomarker of tolerance, hair analysis may be applied to disclose previous drug exposure and thus provide an estimate of the degree of tolerance. The aim of this study was to map the past and recent drug use profiles among drug addicts in the Stockholm area who were subjected to a medico-legal examination.

METHODS: We performed segmental hair analysis and compared the results with drug history information obtained from the police, relatives and medical charts as well as with toxicological results in postmortem blood. Out of 210 cases originally investigated, 166 turned out to be drug abusers. These 166 cases were classified as "opiate overdose cases" (O-cases), "control cases" (C-cases), or "miscellaneous cases" (M-cases). Opiate overdose death was based on the diagnosis made by the responsible pathologist. The C-cases comprised deceased drug addicts who, immediately prior to death, obviously were not incapacitated by drugs and the drugs found in their blood were not responsible for their demise. The M-cases also consisted of drug addicts that did not die of opiate overdose, but incapacitation could not be excluded. Most of these subjects died of natural diseases or conditions secondary to drug abuse.

RESULTS: Hair and blood analysis revealed extensive polydrug use, which was more pronounced among opiate overdose victims (O-cases) than in drug addicts dying of other causes (C- and M-cases). This is evident from Figure 1 which illustrates percent cases in each



group with three or more drugs present in hair (black bars) and blood (grey bars). Blood analysis also showed that pure heroin intoxication was very rare, but typically the addicts presented with a number of drugs at the time for their demise. Segmental hair analysis revealed that in more than 80% of the fatal opiate overdose cases, no opiates were found in the most recent hair segment, supporting the notion that abstinence is an important risk factor for opiate overdose death. The toxicological results further suggest that opiate overdose death is more likely to occur if opiates are combined with benzodiazepines, but less likely if opiates are combined with stimulants such as amphetamines.

A substantial overlap in mean blood morphine concentrations were observed between subjects with evidence of recent period of abstinence compared with subjects showing a continuous opiate use and neither was there a difference between cases with or without ethanol present in blood.

CONCLUSIONS: Our study provides extensive support for abstinence as a major risk factor contributing to acute opiate deaths, and that polydrug use as revealed by both hair and blood analysis, proved to be very frequent.

The Potential of Oral Fluid as a Specimen in Various Drug Testing Programs

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The purpose of this study was to determine the utility of oral fluid as a specimen for drug testing in various areas, such as traffic safety, workplace programs, pain management, and school drug testing. While saliva testing offers advantages over urinalysis, specifically ease of collection and difficulty of adulteration, its implementation on a routine basis has been hampered by insufficient or unknown volume, and lack of sensitivity of detection methods. In this report, neat oral fluid (1 mL) is collected, using a Quantisal™ (QS) device, which dilutes the specimen with 3 mL of transportation buffer. Adequate volume is then available to screen for alcohol and multiple drug classes, at relevant concentrations using ELISA. Sufficient volume remains for the confirmation of many drug classes assuming multiple positive screens were obtained. Confirmatory assays were performed using GC-MS, GC-GC/MS or LC-MS/MS.

As procedures for extraction and analysis improve in efficiency and sensitivity, oral fluid can be used as a specimen for various areas of drug testing.

Drug	Screen (ng/mL)	QS (µL)	Confirmation profile	Cut-off LOQ (ng/mL)
Amphetamine Methamphetamine	50 50	10 10	AMP, MDA METH, MDMA, MDEA	50
Benzodiazepines	10	40	Bromaz; Clonaz; Flunitraz; Nitraz; Alprazolam; Fluraz; Triazolam; Loraz; Oxazepam; Diazepam; Temaz; Chlordiazepoxide; Nordiaz; Midazolam	1
Cannabinoids	4	50	THC; THCA	THC 2 ng/mL; THCA 5 pg/mL
Carisoprodol	100	10	Carisoprodol; meprobamate	50
Cocaine	20	40	Cocaine / BZE	8
Dextromethorphan	50	25	Dextromethorphan, Dexorphan	10
Fluoxetine	50	60	Fluoxetine; Norfluoxetine	25
Opiates	40	10	Morphine, codeine; 6-AM	40; 4
Methadone	50	10	Methadone	25
Barbiturates	50	10	Barbiturate group	50
Phencyclidine	10	10	Phencyclidine	10
Sertraline	50	100	Sertraline; Desmethylsertraline	25
Buprenorphine	Unk-nown		Buprenorphine	0.5
Meperidine	50	40	Meperidine	25
Oxycodone	25	25	Oxycodone	20
Tramadol	50	40	Tramadol; N-DM tramadol; O-DM tramadol	25
Propoxyphene	10	10	Propoxyphene	10
Zolpidem	10	10	Zolpidem	10
Alcohol	0.02%	20	Alcohol	0.02%
	Total: 470 µL			

Standardized Saliva Collection as a Basis for Reproducible Testing in Oral Fluid

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AIMS: When using saliva for diagnostic purposes a number of major problems must be avoided during the collection process: The volume of collected sample should not be subject to high variability and analytes should not be unspecifically absorbed on or

in the collection device as precondition for a reliable saliva testing. A new commercially available saliva collection system was evaluated which is based on an oral cavity extraction fluid, allowing a standardized and reproducible collection procedure of oral fluid. To evaluate the reproducibility of this sampling process saliva from healthy individuals was obtained by this method and the total amount of collected oral fluid was quantified as well as the extracted saliva volume, pH value and amylase activity. To demonstrate a practical application of the system, cotinine was analyzed in the collected oral fluid with the goal of discriminating smokers from non-smokers.

METHODS: 176 healthy control individuals used the Saliva Extraction System (Greiner Bio-One) for saliva collection by rinsing the oral cavity with 4 mL of the Saliva Extraction Solution – SES (pH 4.2), which contains a food dye as internal standard. An evacuated saliva transfer tube is then plugged directly onto the lid of the collection beaker, which allows a direct transfer of the obtained oral fluid in a closed circuit. In the following, the total amount of the collected fluid was determined by weighing, the proportion of harvested saliva by photometric measurement of the dye concentration and calculation of the dilution factor, using Saliva Quantification Kit calibrators. The pH was determined by direct measurements with a pH-electrode and further with a photometric pH reagent on an AU400 Clinical Analyzer from Olympus. Amylase enzyme activity was quantified in the oral fluid samples using the Olympus urine amylase reagent and cotinine by the immunological cotinine urine test from Microgenics.

RESULTS: The tested donors consisted of 78 males and 98 females; the median age was 28 years. Using the described collection method the individual fluid volume collected ranged between 4.4 mL - 9.8 mL (median 6.6 mL) with a saliva content of 34% - 93% (median 62%). Analytical precision is important to accurately calculate the amount of collected saliva. Saliva low controls (30% saliva fraction) showed a CV of < 1.9% compared to a CV of < 0.7% for a high saliva control sample (70% saliva fraction). When rinsing the oral cavity with a SES buffered to pH 4.2, the resulting pH values in the collected samples ranged from 4.7 to 5.6 (median: 4.9) showing good correlations with the photometrically determined pH values. Amylase activity in the collected oral fluid was found to vary inter-individually significantly between 4,000 u/L and 600,000 u/L (median: 78,000 u/L). Studying the amylase stability in the samples, no changes have been observed so far even after repeated freezing and thawing cycles. With the modified urine assay cotinine concentrations were found in the range between 0 to 281 ng/mL, showing a good association to the self-declared smoker versus non-smoker status.

CONCLUSIONS: Using the saliva collection principle based on a buffered liquid rinsing system under defined setup conditions allows a reliable, hygienic and reproducible oral fluid collection. A standardized pre-analytic sampling procedure is the precondition for reproducible quantitative routine determinations of analytes in saliva, which could be demonstrated by the evaluated parameters.

Non-biological analysis of drugs
Analyses de stupéfiants (drogues de saisies)

Evaluation of Erectile Dysfunction Treatment Drugs Obtained Inappropriately in Japan

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AIMS: To maintain anonymity, many men obtain erectile dysfunction (ED) treatment drugs through the internet. However, it has been reported that hundreds of thousands of counterfeit ED treatment drug tablets were seized in Japan in 2006. The present study investigated the authenticity of sildenafil citrate that could be obtained through the internet without prescription in Japan.

METHODS: In October 2006, loose Viagra tablets were obtained from four 'personal import agents' operating Japanese internet websites. The 4 samples obtained were examined by IR spectroscopic and high-performance liquid chromatographic (HPLC) analysis and by courtesy of the manufacturer (Pfizer Ltd), compared with authentic Viagra.

RESULTS: IR spectroscopic analysis showed that samples-1, -2, -3 and -4 were confirmed to contain sildenafil citrate, which is the active pharmaceutical ingredient for Viagra. However, the formulations seems to be different from that of authentic Viagra, because of different IR spectra around 3300 cm-1. HPLC analysis showed samples-1, -2, -3 and -4 contained 88%, 88%, 89% and 106% active ingredient of authentic 100 mg tablet, respectively.

CONCLUSIONS: With the spread of the internet, the amount of Viagra imported without prescriptions are estimated to increase sharply. A considerable number of these tablets seem to be counterfeit produced under poor hygienic conditions. In order to ensure that patients have safe and effective medicines, regulators, pharmaceutical companies, and physicians should enlighten patients to avoid purchasing them through the inappropriate routes.

Simultaneous Analysis of Opioid Agonists; Mitragynine, 7-Hydroxymitragynine and Other Alkaloids in a Psychotropic Plant "Kratom" (Mitragyna speciosa) by LC-ESI-MS

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AIMS: The leaves of a tropical plant, Mitragyna speciosa (known as "Kratom") have been traditionally used as a substitute for opium in Thailand and Malaysia. Mitragynine, a major constituent of M. speciosa, has an opioid agonistic activity and its derivatives, 7-hydroxymitragynine (a minor constituent), shows a much more potent effect than the effects of mitragynine and morphine. While the use of this plant has been banned in the above countries, many kinds of products containing this plant have been recently distributed as "incense" in the drug market in Japan in expectation of its narcotic effect. In this study, to investigate the trend of such non-controlled psychotropic plants of abuse, a simultaneous analytical method was developed using LC-ESI-MS for the active components of M. speciosa; mitragynine, 7-hydroxymitragynine and other alkaloids. Moreover, this method was applied to the analyses of these compounds in the products of M. speciosa.

METHODS: Thirteen products of M. speciosa (pieces of dried leaves, powder and gum), which were advertised as having psychotropic/psychoactive effects, were purchased via the Internet. The products and raw materials (dried leaves) of M. speciosa were extracted with methanol under ultrasonication. After centrifugation, the extracts were filtered thorough a 0.45-μm membrane filter prior to the injection for the LC-MS analysis. The separation of the target compounds, mitragynine, 7-hydroxymitragynine and other alkaloids (speciogynine, speciociliatine and paynantheine) was optimized on an ODS column (Atlantis dC18, 2.1 x 150 mm, 5 μm) in an acetonitrile-10 mM ammonium formate buffer (pH 3.5) by a linear gradient program and a quantitative analysis was carried out by the monitoring of each $[M+H]^+$ in the positive ion mode of ESI-MS.

RESULTS AND CONCLUSIONS: As a result of the LC-ESI-MS analysis, mitragynine, 7-hydroxymitragynine and other alkaloids were found in 12 of the 13 products, the same as in the raw materials. However, one product investigated in this study did not reveal any alkaloids. The contents of mitragynine in the products were in the range from 1 to 6 % and those of 7-hydroxymitragynine were from 1/300 to 1/100 of the mitragynine contents. 7-Hydroxymitragynine has a significantly potent narcotic activity even if compared with morphine. Therefore the abuse of M. speciosa is causing concern. This analytical method could be useful for surveying the distribution of the products of M. speciosa in the drug market.

A Herbal Medicine Adulteration with Sibutramine

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AIMS: Recently, the use of different remedies for body weight loss has widely expanded. Very often they are self-administered without any medical control. Only a few prescription medications to support weight reduction are available in Poland. Many preparations acting as anorectics can however be easily bought via the Internet. Very often they are advertised as herbal supplements, well tolerated, and very potent. In 2006 many reports about developing different symptoms after short-term self-administration of a Chinese medicine, declared as herbal, called Meizitang (MEIZ) were noted. A composition of MEIZ capsules was therefore examined.

METHODS: Unfortunately no biosamples were obtained with only three original packing preparations (with remaining capsules) available for examination. Organic ingredients in metanolic solutions of white powder of MEIZ capsules were identified by GC-MS, LC-MS-APCI, both in TIC and SIM mode, and LC-MS-MS-ESI methods. In the last method chromatographic separation was obtained using a Waters Alliance Separations Module with a LiChrospher RP-18e (125 x 3 mm), gradient elution with a mobile phase of 0.1 % (v/v) formic acid in acetonitrile and water and a total flow of 0.8 mL/min. The detection of sibutramine (SIBU) was carried out using a Micromass Quattro Micro LC-MS-MS system. Two MS-MS reactions together with the surviving ions were monitored for identification: m/z 280 ∀ 125, 280 ∀ 139 and 280 ∀ 280. These and further multi-direction analyses were carried out and compared with Meridia (MERI), a pharmaceutical authorised preparation by Abbott. For pesticide residues the LC-MS/MS method by Alder et al. (Alder L., Kempe G., Vieth B., Mass Spectrometry Reviews, 2006, 25, 838-865) was used. Heavy metal content was determined using flame AAS, cold vapour AAS (applied for Hg) and Sanger-Black (for As) methods after microwave digestion.

RESULTS: One major component was detected and identified as SIBU, an amphetamine related compound. Other organic compounds including demethylated products of SIBU and pesticide residues were not detected. Quantification by LC-MS-APCI method yielded 27.5 mg SIBU base, which was approximately two times higher than in MERI, which contained declared 15 mg SIBU hydrochloride. The average heavy metal content (expressed in μg per capsule) in MERI and

MEIZI capsules were as follows: Cd 0.30 and Zn 0.19 in each; Cr 1.70 and 1.66, Hg 0.006 and 0.02, Mn 0.45 and 0.40, Pb 2.1 and 2.0, Cu 0.68 and 0.67, respectively; and all had no toxicological significance. Additionally, MERI and MEIZI were similar in capsule mass (0.58 g and 0.63 g), but different in colour (blue and grey-orange) and fillery (lactose and starch). Consumers of MEIZ, mostly women, took one capsule of the examined products per day, for approximately one week. On the second or third day they developed severe headaches, dizziness, insomnia, anxiety, oral dryness and metallic aftertaste. After discontinuing administration side effects disappeared within 2 days. In two other cases, when administration was longer, hospitalization was needed because of psychosis. All observed symptoms and others such as tachycardia, hypertension, heart palpitation, nausea and liver disfunction are related to SIBU therapy.

CONCLUSIONS: The described facts demonstrate a prevalent problem concerning herbal medicines, where adulterations with synthetic therapeutic substances can lead to severe adverse effects.

Regioisomers of 3,4-Methylenedioxy-N-methylamphetamine in Clandestine Ecstasy Pills

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AIMS: The drug of abuse 3,4-methylenedioxy–methamphetamine (3,4-MDMA) is one of a total of ten regioisomeric 2,3- and 3,4-methylene–dioxyphenethylamines of molecular weight 193 and fragment ions with equivalent mass (m/z: 58 and m/z: 135/136) in the electron impact mass spectrum. Some of these regioisomers (prepared in laboratories using the most popular synthesis methods used in clandestine manufacture) have been studied to determine whether any of them pose a potential problem for the proper identification of 3,4-MDMA or whether they could be mistakenly identified in routine analytical procedures used in drug testing laboratories. The aim of this paper is to determine the nature of the contaminants and/or adulterations present in ecstasy pills. This study focuses on the cases in which the possible existence of regioisomers of 3,4-MDMA was observed.

METHODS: Different analytical techniques were applied to 28 ecstasy pills seized by the police in and around the city of Valladolid (Spain). Legal authorization and ethical approval was obtained. All the samples analysed showed up positive for MDMA in the Marquis test. In the gas chromatography coupled to mass spectrometry (GC-MS) analysis, 9 out of the 28 samples showed a delay in the peak retention time corresponding to the MDMA. The chromatogram allowed us to discover an overlapped peak at retention time of MDMA, in which it was possible to see the existence of two or more compounds depending on the sample in question.

RESULTS: Mass spectrometry confirmed, in the different zones of the uncoupled peak of each of the 9 samples, the existence of a compound with a molecular ion m/z: 193 and the fragment ions showing the presence of MDMA in the samples. The molecular weight 193 was confirmed using chemical ionization (CI-MS) with methane gas, where an intense ion $[M+H]^+$ m/z: 194 was observed in all cases. On the other hand, in the high performance liquid chromatography (HPLC), using photodiode and fluorescence detectors in line, no peaks were observed that could correspond to the uncoupling seen in the gas chromatography. The spectra registered by the photodiode detector between 200 and 400 nm were constant in the peak. However, in the MDMA quantification using the compound 3,4-MDMA as the external standard (chromatograms obtained at 285 nm with the PDA detector or with the fluorescence detector at an excitation wavelength of 285 nm and emission wavelength of 320 nm), concentrations over 100% were found of the active element (3,4-MDMA) in the pills in which overlapped peaks in the chromatogram were apparent. This could be explained by the presence in the mixture of compounds with a greater absorbance than the standard at the wavelengths used in the analyses.

CONCLUSIONS: The analytical results indicate that the samples, in which an overlapped peak assigned to MDMA was observed, contain 2 or more of the 10 regioisomers that exist in MDMA. The presence of one, or several, of these regioisomers can easily be missed in routine analyses and can lead to an important error of quantification. This study was carried out by support of Consejería de Educación, Junta de Castilla y León VA115/04. This study was also supported by a grant from Redes Temáticas de Investigación Cooperativa, Red de Trastornos Adictivos, RD06/0001, Instituto de salud Carlos III

The Use of Raman Spectroscopy to Profile Seized Ecstasy Tablets

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AIMS: The use of Raman spectroscopy with near infrared excitation (785 nm) applied to the analysis of ecstasy tablets is well documented. High sample throughput is essential if a technique is to be useful

for operational analysis and intelligence gathering applications. Clandestine laboratory seizures often comprise several hundred thousand tablets and composition analysis of active drug and excipient components is required. The aim of this work was to determine if Raman spectroscopy could be used to distinguish between batches of tablets containing chemically similar ring-substituted phenethylamines and multiple excipients, to give useful composition profiles for drug intelligence work. Ecstasy tablets, seized from clandestine laboratory raids, were obtained from the Federal Police in Belgium.

METHODS: The tablet was mounted on a microscope slide and placed under the microscope for Raman spectral collection using a Renishaw Raman Microscope equipped with a 785 nm laser. Typically, the laser was line focused onto the sample enabling collection of a series of line images. Spectra were collected from three areas (top, middle and bottom) on the tablet to maximise the total area sampled and to compare any similarities and or differences across the tablet. The total analysis time per tablet was 15 minutes. Reference standard spectra were collected for comparison.

RESULTS: The spectral similarities between MDMA, MDA and MDE when present as a mixture make it difficult to assign the presence of a particular drug. This is compounded by the crude methodology used to manufacture the tablets, resulting in the presence of multiple derivatives rather than MDMA itself, which is typically sought. Additionally, the smearing effect of the tablet casting machines may amalgamate the particles further complicating the spectral representation. Consequently, the phenethylamine derivatives peak positions were averaged to facilitate comparison with the identified excipients. Interestingly the disparities in peak positions identified in the phenethylamine derivatives were not observed with the excipients (glucose, sucrose, cellulose and sorbitol). It is thought this might be related to the quality control in production of these by licensed manufacturers. Analysing three areas per tablet and averaging the peak position and peak intensity data gives representative data that can be used to compare tablets within a batch and between batches. The tablets showed variability in the phenethylamine derivatives and excipients across the batches but also revealed trends within the sub-batches.

CONCLUSIONS: Overall this study has shown that ecstasy tablet composition profiling can be undertaken with some degree of success using Raman spectroscopy. Whilst it is not possible to specifically identify the phenethylamine present, using averages of the responses collated from multiple areas on the tablet and comparing these to the excipients can still produce useful data for comparison within batches and between batches. Analysis can be undertaken with virtually no sample preparation with higher throughput than traditional methods.

Impurity Profiling of Amphetamine-Type Stimulant Tablets Seized in Japan by GC-MS

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AIMS: There has been a considerable increase in the number of seizures of amphetamine-type stimulant (ATS) tablets in Japan. Characterization and classification of seized ATS tablets can provide very useful information in criminal investigations aimed at identifying drug traffic routes, the sources of supply and relationship between seizures. In the present study, chemical profiling of organic impurities in ATS tablets was examined by gas chromatography-mass spectrometry (GC-MS).

METHODS: ATS tablets were ground to a powder, and 25 mg of the homogeneous sample was dissolved in 1 mL of 1 M Tris-HCl buffer, pH 8.0. The suspension was vortexed for 5 min and centrifuged. The supernatant was filtered through a membrane filter (0.45 μm). The filtrate was extracted with 0.5 mL of dichloromethane (containing n-docosane as IS). After centrifugation, the lower organic layer was subjected to GC-MS. The column was a DB-5 capillary column (length, 30 m; i.d.; 0.32 mm, film thickness, 1.0 μm). The oven temperature was programmed as follows: 50°C for 1 min, 10°C/min to 300°C. The injector and ion source temperatures were set at 240°C and 230°C, respectively. Helium was used as the carrier gas at a constant column flow-rate of 2 mL/min. Injection of 2 μL of the extract was made in the splitless mode.

RESULTS: In the early stage of this study, optimization was made for extraction conditions of organic impurities in ATS tablets. As pH was increased, extraction efficiencies of basic impurities became better, while those of neutral impurities not impacted. Higher pH also enlarged peaks of main components (MDMA and MDA) in tablets, leading to the overload of samples into the instrument. As a compromise, pH 8.0 was adopted for further experiments. Dichloromethane efficiently extracted impurities under pH 8.0 among organic solvents tested (ethyl acetate, diethyl ether, t-butylmethyl ether, toluene, dichloromethane and 1-chlorobutane). Fifteen impurity peaks were selected for comparative analysis of tablets. Twenty-two samples were classified into at least 3 groups by a hierarchical cluster analysis. One group was composed of MDA tablets, and another 2 were composed of MDMA tablets, which might indicate the different routes of MDMA synthesis.

CONCLUSIONS: Impurity profiling of ATS tablets seized in Japan was examined by GC-MS after optimization of extraction conditions. ATS tablets were classified into at least 3 groups (one MDA and two MDMA groups). The method would provide useful information about relationship between tablets.

Analysis of Drugs and Poisons by LC-TOF-MS: Preliminary Studies on Magic Mushroom Toxins Psilocin and Psilocybin

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AIMS: Time-of-flight (TOF) mass spectrometry (MS) was recognized to be useful especially for high-molecular-weight compounds such as polypeptide, proteins and polysaccharides. During recent several years, resolution ability and sensitivity of TOF-MS instruments have been greatly improved, and the combination of liquid chromatography (LC) and TOF-MS has been realized. In the present study, we have tried to analyze small-molecular magic mushroom toxins psilocin and psilocybin in magic mushroom samples (Psilocybe subcubensis Guzman) by LC-electrospray ionization (ESI)-TOF-MS and/or LC-ESI-quadrupole (Q)-TOF tandem MS. To our knowledge, a few reports on LC-TOF-MS analysis of drugs and poisons in biological samples have appeared.

METHODS: A 20 mg weight of a dried mushroom was mixed with 2 mL methanol and subjected to ultrasonication. The methanolic suspension was centrifuged at 1000 g for 5 min. To the sediment, 2 mL methanol was again added, ultrasonicated and centrifuged. The combined methanolic extract was evaporated to dryness by a centrifugal freeze dryer. The residue was dissolved in 1 mL of the below mobile phase solution. A 5-microliter volume was subjected to the LC-MS or LC-MS/MS analysis.A QSTAR®,^{R}XL hybrid LC-MS/MS system was used for analysis. This system enabled LC-single TOF-MS and LC-tandem Q-TOF-MS. The separation column used was Inertsil ODS-3 (2.1 x 150 mm, particle size 5 micrometer). The mobile phase was 10 mM ammonium formate (pH 3.5)/ methanol (80:20). Isocratic elution was made at a flow rate of 0.2 mL/min. Under our conditions, psilocin (m/z 205) and psilocybin (m/z 285) appeared at the retention times at 5.2 and 3.4 min, respectively.

RESULTS: The concentrations of psilocin and psilocybin in a magic mushroom cap were 41.9 and 586 µg/g dry weight, respectively; those in the stem were 44.9 and 534 µg/g dry weight, respectively. Further experiments are now under way.

CONCLUSIONS: LC-TOF-MS(-MS) was found very useful also for analysis of small-molecular psilocin and psilocybin in biological samples, because of high sensitivity and high resolution.

Impurity Profiling of Crystalline Methamphetamine ('Ice') by Automated Solid-Phase Microextraction (SPME) Coupled with Gas Chromatography-Flame Ionization Detection

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AIMS: Impurities of methamphetamine (MA) show different patterns under various conditions of synthesis, and they can be utilized for investigation of their origins and smuggling routes. Generally, there are only traces of impurities in crystalline MA ('Ice'). Specific extraction methods are required to selectively identify impurities in MA. Liquid-liquid extraction (LLE) method has been widely used for impurity profiling for its stability and compatibility, but it is limited by the high concentration of MA that co-extracts. Headspace solid-phase microextraction (SPME) and thermal desorption (TD) methods have been developed as complementary techniques for selective extraction of trace impurities. The aim of this work was to develop an automated SPME method coupled with gas chromatograph-flame ionization detector (GC-FID) to improve reproducibility and reliability of the profiling result.

METHODS: Areas of impurity peaks were normalized by using solid nonadecane (C_{19}) diluted with potassium bromide (KBr) powder as an internal standard. A bi-polar SPME fiber coated with divinylbenzene/ carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was used for extraction of various impurities, and the extraction condition was optimized at 85°C for 20 min after incubation for 5 min. The suitability for impurity profiling was examined by comparing extraction efficiency of the SPME with the LLE method. LLE methods developed by National Research Institute of Police Science (NRIPS) and the United Nations International Drug Control Programme (UNDCP) are most widely used recently for extraction of MA impurities. The NRIPS method has been preferred for impurity profiling of crystalline MA ('ice') and the UNDCP method for MA tablets ('yaba'). The NRIPS method was used in this work.

RESULTS: Intensities of volatile and some of semi-volatile impurities extracted by SPME method were much higher than those extracted by LLE method, and interferences of MA and its artifacts were not observed. Highly reliable profiling results could be obtained by using SPME method for confirmation of similarity, which comes from high selectivity for impurities and

differences in impurity patterns from LLE method. By cluster analysis of 11 MA samples of different origins, 9 samples were classified by both methods, but 2 samples could be classified only by SPME method.

CONCLUSIONS: Improved reliability of the profiling result obtained by SPME method will contribute to efficient investigation on the origins and smuggling routes of MA seizures.

Library-Based Identification of Designer Drugs in Seized Samples by LC-TOFMS and GC-MS

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AIMS: Analysis of seizures for controlled drugs is a regular part of the duties of forensic science laboratories. These investigations often involve the use of a multitude of analytical techniques and a comprehensive collection of reference standards. However, reference standards of designer drugs are not generally available or their supply is hindered by administrative and time restrictions. In this paper, identification of designer drugs in seized samples is carried out without reference standards using two powerful techniques, LC-TOFMS and GC-MS.

METHODS: The LC-TOFMS instrumentation consisted of an Agilent 1100 pump coupled to a Bruker micrOTOF time-of-flight mass analyzer. The identification method was based on an in-house database of one thousand exact monoisotopic masses representing the elemental formulas of toxicologically relevant drugs, including the data from PiHKAL and TiHKAL. The GC-MS instrumentation consisted of a HP 6890 Plus gas chromatograph coupled to a Hewlett-Packard 5973 mass selective detector. The GC-MS method was based on four extensive commercial libraries containing electron ionization spectra. We used the newest versions available: NIST/EPA/NIH Mass Spectral Library 2005 (NIST05) with 190,825 spectra, Wiley Registry of Mass Spectral Data 7th Edition with NIST 2005 Spectral Data (Upgrade) library (Wiley7NIST05) with more than 461,000 spectra, Pfleger-Maurer-Weber Drug and Pestiside Library (PMW-TOX3) with 6,350 spectra and Mass Spectra of Designer Drugs 2006 library (DD2006, created by Peter Rösner) with 5,531 spectra.

RESULTS: Fifteen seized samples received from the Finnish Customs Laboratory were analyzed by the LC-TOFMS and GC-MS methods. The average number of hits by LC-TOFMS was 2.9 (range 1-9), always including the correct compound. By GC-MS, there were 4 correct #1 hits among the 15 samples with NIST 05, 5/15 with Wiley7NIST05, 3/15 with PMW-TOX3 and 10/15 with DD 2006. Only 2 compounds out of 15 were not included in any of the GC-MS libraries.

CONCLUSIONS: The combined use of LC-TOFMS, based on accurate mass measurement, and GC-MS, based on the extensive commercial libraries, provides a satisfactory means of identifying designer drugs in seized material without reference standards. However, regular updating of the LC-TOFMS database and GC-MS libraries is necessary to cope with the changing drug scene.

Improving Sensitivity in Street Drug Analysis Through an Innovative Ionization Source: Surface Activated Chemical Ionization

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AIMS: This work presents a new ionization source able to significantly improve accuracy and selectivity in street drugs analysis, named Surface-Activated Chemical Ionization (SACI, Cristoni S., Rubini S., Bernardi L.R., "Development and applications of surface-activated chemical ionization", Mass Spectrometry Review 2007), by comparing its results with those obtained by means of commonly employed Electrospray (ESI) and Atmospheric Pressure Chemical Ionization (APCI) sources. SACI grounds its superior performances on a novel ionization mechanism, based on the activation of ionization processes in the presence of a metallic surface, placed at low potential (50 – 600 V), and inserted in an atmospheric pressure ionization chamber. The ionization effect can be attributed to interactions between gas-phase analyte molecules and solvent molecules adsorbed and polarized on the surface by the electric field.

METHODS: A mixture containing morphine, codeine, 6-monoacetylmorphine (6-MAM), benzoylecgonine, cocaine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDE), amphetamine and methamphetamine was analyzed. The molecules were separated by reverse phase chromatography before mass spectrometry analysis. A C18 50 x 2.1 mm column was used. The eluent phases were: A: H2O + 0.1 % formic acid and B: CH3CN + 0.1% formic acid. A linear gradient passing from 5% to 40% of B in 15 minutes was employed. SACI spectra were obtained using a steal surface placed at a potential of 150 V. The APCI spectra were obtained using a corona discharge current of 4-5 μA. In the case of ESI the needle voltage was 5 kV. The nebulizing gas flow (nitrogen) was 2.5 L/min in all cases. LC-ESI, APCI and SACI mass chromatograms were obtained using the tandem mass spectrometry (MS/MS) approach.

RESULTS: Analytical data and outcomes clearly show that SACI provides a highly sensible approach: LOD and LOQ are respectively 5 and 10 times lower than those achieved by ESI and APCI, with performances between 1-10 pg injected (LOD) and between 5-50 pg injected (LOQ). Linearity range (across 4 to 5 order of magnitude; r^2 between 0.9938 – 0.9985) attests the excellent capabilities of SACI in terms of quantitative analysis. Additionally, the comparison between SACI and commonly employed ionization sources highlights how the factor of improvement in the increase of the signal to noise ratio grows as analyte concentration gets lower, a phenomenon connected to SACI's high ionization efficiency.

CONCLUSIONS: The new SACI approach provides an extremely effective solution for routine drugs screening, in terms of accuracy (even at low analyte concentrations), selectivity, quantitative performances as well as unitary cost per analysis. In particular, the present work shows that SACI is the most sensible approach when compared with ESI and APCI ones, mainly due to the high ionization efficiency and the lower chemical noise of this new ionization source. SACI can therefore represent an excellent solution for routine drugs screening programs.

Drugs of abuse
Substances donnant lieu à abus

Determination of Urine Luck in Urine using Electrospray Ionization Tandem Mass Spectrometry

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AIMS: Inductively coupled plasma (ICP) mass spectrometry (MS) is often used for the determination of chromium (Cr). This method, however, cannot distinguish between chromate (Cr^{6+}) and chromium (Cr^{3+}). The selective determination of Cr^{6+} is required since Cr^{6+} is more toxic than Cr^{3+}, and only Cr^{6+} is present in adulterants such as Urine Luck that may conceal tetrahydrocannabinol and morphine present in urine. Previously, we reported the application of electrospray ionization (ESI) MS for the determination of Cr^{6+} after complex formation with diethyldithiocarbamate (DDC, $(C_2H_5)_2NCSS$-). The sample volume required in ESI-MS is one hundredth of that required in ICP-MS, although the LOD in ESI-MS is 3 times higher than that in ICP-MS. In the present study, selected reaction monitoring (SRM) in ESI-MS/MS was applied to lower the LOD using a minute urine sample (10 µL) without a concentration process.

METHODS: Cr^{6+} was complexed with DDC and extracted with isoamyl alcohol in the presence of citric acid. The detection of Cr^{6+} was achieved by injecting 1 µL of the isoamyl alcohol containing Cr-DDC complex directly into a TSQ 7000 MS/MS (ThermoQuest, Japan) without chromatographic separation. The three quadrupoles served to isolate the precursor ion, function as a collision cell and separate the product ions, respectively. The complex was ionized at 4.5 kV using ESI in the positive mode. The characteristic spectrum appeared 30 s after sample injection, and a sample could be injected every 30 s. Methanol was used as a mobile phase and the oven temperature was set at 280°C. The MS/MS data were collected in the range of m/z 50-550 with a scan time of 1 s. Quantification was performed using SRM at m/z 513.1 ± 0.3 of precursor ion, $CrOH(DDC)_3$+ and at m/z 363.8 ± 0.2 of product ion, $CrO(DDC)_2$+ after collision-induced dissociation at 16 V.

RESULTS: This method was validated for the analysis of urine samples; the LOD and LOQ were 0.05 and 0.18 µg/L, respectively, using only 10 µL of urine. The concentrations of Cr^{6+} determined from the peak area of mass chromatogram (y) were proportional to the concentrations spiked (x) up to100 µg/L, $y = 0.9999 x + 0.3171$, with a correlation coefficient of 0.9989 in urine. Precision and accuracy were assessed by the analysis of urine and 0.15 M NaCl solution containing 1 mM HNO_3 spiked at 0.1, 1, 10 and 100 µg/L, respectively. These samples were analyzed three times a day as well as on three different days. The CV was < 19%, and accuracy was between 85 to 119% for both intra-day and inter-day variations in any sample - even urine spiked at 0.1 µg/L. After the oxidation of Cr^3 to Cr^{6+}, the complete recovery was observed in the standard reference materials such as SRM 2670a and SRM1643e.

CONCLUSIONS: The ESI-MS/MS method described provides more valuable information for the identification of compounds and less matrix interference than single GC-MS methods.

Urine Analysis in Patients of a Heroin Maintenance Program Compared to Participants of a Methadone Maintenance Program

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AIMS: Urine samples from participants taking part in a heroin maintenance program (HMP) and a methadone maintenance program (MMP) were analysed mainly to compare additional consumption of illicit (street) heroin. Analytes of interest were morphine (MOR), morphine-3-glucuronide, morphine-6-glucuronide and 6-monoacetylmorphine (MAM) as metabolites of

pharmaceutical heroin, as well as 6-acetylcodeine (AC), codeine (COD), codeine-6-glucuronide, noscapine (NOS) and papaverine (PAP) as markers of illicit heroin.

METHODS: One month before (T-1) as well as 6 (T-6) and 12 (T-12) months after controlled administration of pharmaceutical heroin-HCl (10 - 100 mg/d) or methadone (15 - 260 mg/d) urine samples of patients were analyzed using a validated LC-MS procedure. For other drugs of abuse (cannabinoids, amphetamines, cocaine metabolites, benzodiazepines, methadone) immunochemical procedures were used.

RESULTS: Limits of detection were between 0.1 ng/mL (NOS) and 7.4 ng/mL (MOR-glucuronides). Coefficients of correlation were higher than 0.99, precision varied between 4 - 12%, and absolute recoveries ranged from 40% (MOR-glucuronides) to 97% (COD-6-glucuronide). In urine samples taken from opiate addicts one month before the program's start MOR and MOR-glucuronides were found in 100% of cases, often with simultaneous detection of MAM (85%), AC (86%), COD (93%), COD-6-G (96%), NOS (94%) and PAP (87%). Urine analyses at T-6 and T-12 revealed the following results for an additional abuse of illicit heroin: HMP 16.4 % positives at T-6 and 15.0 % positives at T-12; MMP 35.4% positives at T-6 and 30.0% positives at T-12. Also the additional cocaine use decreased more markedly in the HMP compared to methadone substitution, other substances showed no significant differences.

CONCLUSIONS: The method's applicability was proven by analysis of authentic urine samples. Consumption of illicit heroin significantly decreased during application of pharmaceutical heroin compared to a MMP. The difference in additional cocaine consumption at T-12 was not statistically. Patients in the HMP show a decreased concomitant drug abuse compared to participants in a MMP.

Update on Epidemiology of Substance Abuse

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AIMS: Knowledge of substance abuse trends is essential in forensic toxicology and DUI enforcement. The aim of this presentation is to review and summarize the highlights of the most recent reports of the major substance abuse epidemiological tools.

METHODS: Data from several sources were used to summarize the current epidemiology of substance abuse.

RESULTS: Drug Abuse Warning Network: Of 106 million US emergency department (ED) visits in 2004, 2% were drug related with 19% of those related to cocaine, 8% to heroin & 5% to stimulants; 1.2% of ED visits were drug abuse/misuse related with 30% from illicit drugs only and 38% from non-medicinal use of prescription or OTC drugs. Monitoring the Future: (8, 10, 12th Grades) Use of any illicit drug in past 12 months has decreased since the last report but 50% in 12th grade have tried illicit drug(s). THC has been most prevalent illicit drug with peak use at 50% in late 70's and now near 40%. Most illicit drugs have experienced a decrease in prevalence of lifetime use while cocaine and heroin have held steady. Increased use of sedatives, oxycontin and inhalants are reported.
National Survey on Drug Use & Health: Among persons ≥ 12 yrs, 8.1% reported recent illicit drug use and 2.6% reported nonmedical use of prescription-type psychotherapeutic drugs (3/4 of which were pain relievers). Youth Risk Behavior Surveillance System: (Grades 9-12) These data provided trends from 1991 to 2005 as follows: drove while drinking ETOH 16.5 to 9.9%; episodically drink ETOH heavily 31.3 to 25.5%; currently use - THC 14.7 to 26.7 to 20.2%; cocaine 1.7 to 3.4%; lifetime use - inhalants 20.3 to 12.4%; steroids 2.7 to 6.1 to 4%. For the years 1999 to 2005 the following trends were observed: methamphetamines 9.1 to 7.6 to 6.2%; years 2001-2005 ecstasy 11.1 to 6.3%, hallucinogens 13.3 to 8.5.

CONCLUSIONS: Most illicit drug use has decreased in recent years in the US but non-medicinal use of prescription drugs, in particular pain relievers has increased.

Determination of Cocaine, Benzoylecgonine and Anhydroecgonine Methyl Ester in Urine Specimens from Individuals Submitted to Medical-Legal Investigations

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AIMS: Smoking cocaine (COC) has become popular in recent years, mostly in North American and some Latin American countries mainly due to its rapid delivery of drug to the brain. Smoking also causes an intense craving for additional drug. When cocaine is smoked, anhydroecgonine methyl ester (AEME) is also inhaled as a pyrolytic product, which can be used as an analytical marker for crack smoking. The objective of this study is the development and application of a method that is efficient and economically viable for the identification and quantification of crack biomarkers in urine samples.

METHODS: We developed a gas chromatography-flame ionization detection (GC-FID) method for the identification and quantification of AEME, benzoylecgonine (BE) and COC in urine. The analytes were extracted from urine using a routine solid-phase extraction procedure. The method was evaluated for the

production of AEME as an artifact by fortifying COC stock solution (1g/L) to negative urine, in triplicate, at 0.5, 2.0, 4.0, 5.0, 50 and 100 µg/mL. The injector of the GC-FID was maintained at 230ºC, the sample was analyzed and the production of artifact monitored by formation AME. The method was applied to positive urine specimens obtained from 13 postmortem and 24 antemortem cases. Screening was performed using thin layer chromatography and immunoassay and positive cases were confirmed by GC/FID and GC/MS.

RESULTS: Method validation parameters were as follows:

Parameter	AEME	BE	COC
Limit of detection; mg/L	0.1	0.05	0.05
Limit of quantitation; mg/L	0.2	0.1	0.1
Linear Range; mg/L	0.2 – 4.0	0.1 – 3.0	0.1- 5.0
Correlation coefficients, r^2	0.9979	0.9934	0.9977
Recovery QC's (3.0, 1.0, 0.5 mg/L); %	90, 79, 63	85, 82, 73	99, 98, 96
Accuracy QC (3.0 mg/L) n = 6 days; %	83	89	89
Within-day precision QC (3.0/mg/L) n = 6; %	10.26	13.10	2.20
Between-day precision QC (3.0 mg/L) n = 6 days; %	14.45	11.73	10.40

Negative urine was free from interfering agents as determined by GC-FID. Artifactual production of AEME could be excluded in urine with COC concentrations between 0.5 and 50 mg/L. The AEME artifact was present in urine with COC concentrations of 100 mg/L but below the limit of detection. AEME was detected in 3 of 13 (23%) postmortem specimens and 8 of 24 (33%) antemortem specimens indicating that smoking is the important route of administration COC. BE was present in large concentrations in all urine samples.

CONCLUSIONS: A method for the detection of AEME, BE and COC by GC-FID was developed in urine. The limit of detection and quantification were adequate for the concentrations of AEME, BE and COC normally seen in urine. AEME was not produced as an artifact at relevant concentrations. Although BE is a better biomarker to identify COC use, AEME is an excellent marker for documenting use of COC by the smoked route. The method was found to be efficient in discriminating between smokers and non-smokers of cocaine and can be used for the quantification of higher AME concentrations in forensic cases.

Alcohols and alcohol biomarkers
Alcool et marqueurs biologiques de l'alcoolisme

Methanol as an Alcohol Abuse Marker in Forensic Samples

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AIMS: Alcoholism is one of the main health problems in the world; therefore, detecting this condition is important in legal, labor and health areas. Different substances have been proposed as alcoholism markers, among which methanol stands out because of the availability of standards and because most of the forensic laboratories have reliable techniques to determine its presence. Several contributors have studied the usefulness of methanol as an alcoholism marker in samples obtained from living people. However, to date no work has been carried out in postmortem samples.

METHODS: In this study, methanol in postmortem samples from alcoholics with and without ethanol present at the time of death, social drinkers and teetotallers (negative control group), was determined with gas chromatography with headspace injection to study the possibility of using methanol as an alcoholism marker in postmortem samples.

RESULTS AND CONCLUSIONS: As expected, a higher methanol concentrations were found in samples from alcoholics positive for (Table 1), followed by social drinkers and alcoholics negative for ethanol. All of these groups had significantly more methanol than the teetotallers group.

Table 1: Methanol and ethanol concentrations (mg/dl) in the four groups studied

Studied Groups	Teetotallers (n = 94)	Social drinkers (n = 22)	Alcoholics without ethanol (n = 28)	Alcoholics w/ ethanol (n = 42)
MeOH mean (SD)	0.363 (0.338)	3.391 (3.200)	2.039 (2.162)	6.051 (10.252)
MeOH range	0-1.544	0.705-12.188	0 – 10.458	0.453-62.062
EtOH range	< 20	42 – 362	< 20	27 - 368

After eliminating some abnormally high values from people suffering metabolic disorders like diabetes in the teetotallers group, a normal distribution was found allowing this group to be considered as a negative control (x = 0.313 mg/dl, SD = 0.248 mg/dl). In contrast to controlled studies in living subjects, more methanol was found in postmortem specimens in all groups studied. In addition, the distribution of results was less homogeneous.A weak correlation (r = 0.4179) between methanol and ethanol was found in social drinkers suggesting that methanol found in this group

came partially from alcoholic beverages. A similar correlation was not found (r = -0.047) in the alcoholic group. Methanol concentrations in the social drinkers were not statistically different from methanol found in the alcoholic groups, therefore when ethanol is present the specificity of methanol as marker for alcoholism low and consequently could be used as a valid alcoholism marker only when ethanol is not present. Methanol concentrations in alcoholics negative for ethanol were particularly heterogeneous ranging for negative to high. These reflect the end of ethanol elimination and different stages of methanol elimination and can be related to ethanol withdrawal and the time since ethanol was last consumed.

Disappearance Rate of Ethanol from the Blood and the Breath of Healthy Humans: The Influence of Food and Fructose-rich Soft Drinks

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AIMS: Fructose-rich nonalcoholic drinks often appear on the market, advertised by the producer as effective in speeding up the rate of alcohol elimination, potentially yielding a four-fold increase in the rate of ethanol metabolism. To establish this, and to account for food-induced differences, various drinking experiments were designed.

METHODS: Nine healthy, 23–24 year-old nonalcoholic subjects were studied: 4 men and 5 women. All subjects received ethanol (1 g/Kg) on two occasions: orally as spirits after an overnight fast, and orally as wine during a rich dinner. A 43 g quantity of fructose in the form of a soft drink was given to the subjects in the post-absorptive phase. Ethanol was measured in expired air and in venous blood every 30 min, 120 min after the end of ingestion, by Dräger Alcotest 7410 and by headspace gas chromatography, respectively. The beta-slope of the alcohol curve (the elimination rate of alcohol from the breath and blood in the post-absorptive phase) was monitored.

RESULTS: Orally ingested ethanol in the fasting state produced a slower elimination rate (Blood: average 0.107 g/Kg/h, men 0.100 g/Kg/h, women 0.114 g/Kg/h; Breath: average 0.119 g/Kg/h, men 0.111 g/Kg/h, women 0.126 g/Kg/h) than in the feeding state (Blood: average 0.152g/Kg/h; men 0.146g/Kg/h, women 0.157g/Kg/h; Breath: average 0.179 g/Kg/h, men 0.156 g/Kg/h, women 0.191g/Kg/h). A fructose-induced increase in the rate of elimination of ethanol was also confirmed when subjects consumed a fructose-rich beverage 2 hours after drinking, that is, when the post-absorptive phase of ethanol metabolism was well established. An increased rate of elimination of alcohol from the blood and breath was observed in feeding subjects of between 22 and 62% (0.22–0.26 g/Kg/h, average: 0.23 g/Kg/h) and between 28 and 55% (0.14–0.18 g/Kg/h, average: 0.16 g/Kg/h) in overnight fasting subjects, but the effect lasted only for one hour.

CONCLUSIONS: The data confirm the influence of feeding state and gender, but fail to confirm that of fructose in the manner claimed by the producer on blood ethanol response after a moderate dose of ethanol. These data suggest caution for drinkers, given the short-lasting effect of fructose sobering drinks.

Confirmatory Analysis of Ethylglucuronide and Ethylsulphate in Urine by LC-MS/MS According to Forensic Guidelines

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AIMS: Ethylglucuronide (EtG) and Ethylsulphate (EtS) are stable Phase II metabolites of ethanol that can be detected in urine samples several days after elimination of ethanol. Determination in urine is mainly performed by LC-MS, LC-MS/MS, or by GC-MS. This paper describes a new, fast, easy and reliable method to detect EtG and EtS in urine samples by dilution and direct injection into an API 3200™ LC-MS/MS system. The method was validated according to forensic guidelines and tested for possible false positive results due to consumption of alcohol containing food or use of alcohol containing personal care products.

METHODS: An LC-MS/MS method has been developed to detect the following Multiple Reaction Monitoring (MRM) transitions: deprotonated molecule of EtG [M-H]- to product ions m/z 75 and 85 and EtS [M-H]- to m/z 97 and 80. Isotopically labeled internal standards were used to evaluate ion suppression effects. Simple dilution with water containing 0.1% formic acid followed by centrifugation was found to be sufficient to prepare urine samples. HPLC separation was performed on a Phenomenex Synergy Polar-RP (250 x 2.1 mm) column using a gradient of water, acetonitrile and formic acid. The flow rate was 200 µL/mn. Post-column addition of acetonitrile at 200µl/mn was used to enhance sensitivity.

RESULTS: EtG was detectable at concentrations below 1 µg/L and EtS below 0.1 µg/L. No carry-over causing possible false positive results could be observed analyzing urine samples. Linear regression with 1/x weighting was used resulting in accuracy over the

complete linear range of 90 - 110%. The coefficient of variation (%CV) was found to by less than 15% over the complete linear range with a number of 10 injections at each level. The %CV of the ratio of both MRM transitions of each analyte was below 6%. Volunteers participated in studies to simulate various consumption scenarios of e.g. airline pilots, machine operators and patients undergoing alcohol withdrawal programs. Urine samples were collected before, during, and after consumption and kept refrigerated prior to LC-MS/MS analysis. Time plots are used to study the kinetics of metabolism of ethanol.

Comparison of a Direct Immunoassay (N Latex CDT) and Capillary Electrophoresis for the Determination of Carbohydrate-Deficient Transferrin

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AIMS: In several scientific investigations, a strong relationship between increasing blood alcohol concentration (BAC) in drivers and increasing risk of road accident has been irrefutably shown, in particular the seminal Grand Rapids study. Objective laboratory evidence of heavy drinking is needed in medico-legal cases for identifying individuals with alcohol problems and for monitoring abstinence from alcohol when offenders reapply for a driving license. Carbohydrate-deficient transferrin (CDT) is presently considered as the most specific marker of alcohol abuse. Several methods for CDT determination have been described and are used by laboratories. HPLC or capillary electrophoresis (CE) has been recommended because these methods allow separation and identification of the different transferrin isoforms, in particular the CDT.

Recently, a direct immunoassay has been developed and commercialized (N Latex CDT; Dade Behring). This method is based on a monoclonal antibody that measures specifically the transferrin isoforms lacking 1 or 2 N-glycan chains (asialo-, monosialo-, and disialotransferrin). Simultaneously, total transferrin (Tf) is determined in order to express the CDT results as a percentage of Tf. The present study aims at comparing a large number of determinations (n = 538) of CDT by CE and N Latex immunoassay.

METHODS: To separate and measure CDT by CE (CDT: sum of asialo-, monosialo-, and disialotransferrin), a previously described and validated CE method with the Ceofix CDT reagent (Analis) on a Hewlett Packard 3D-CE instrument was used. Subjects were Swiss drivers referred to the Institute of Forensic Medicine because of driving while under the alcohol influence or because of reapplying for a driving license. These subjects were recruited in 2003, 2004, 2006, and 2007. No selection has been made concerning the alcohol consumption of the subjects, resulting in a wide population including teetotalers, occasional drinkers, and regular high alcohol consumers. As observed in other studies dealing with drivers under the influence of alcohol or drugs, males (91%) heavily predominate over females (9%). The mean age of the subjects is 41 ± 13 (S.D.) years.

RESULTS: N Latex CDT results ranges from 0.8% to 10.7%, and 75% of the results are less than 2.3%. CDT CE results ranges from 0.2% to 18.9%, and 75% of the results are less than 1.4%. The results show a good correlation between CE and N Latex CDT ($r^2 = 0.84$; $p < 0.001$), and a good concordance (93%) in terms of positive-negative samples (CE cutoff value: 1.7% and N Latex CDT cutoff value: 2.5%). N Latex CDT results from samples with genetic transferrin variants (n = 9) give similar results to those obtained with CE.

CONCLUSIONS: This study supports the use of N Latex CDT test as a specific enough alternative to CE methods.

Performance Evaluation of High Sensitivity Ethyl Glucuronide Enzyme Immunoassay on the Olympus AU640 Analyzer

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AIMS: The objective of the study was to evaluate performance of the high sensitivity Ethyl Glucuronide (EtG) enzyme immunoassay on the Olympus AU640 analyzer. Ethyl glucuronide is a stable, non-volatile direct metabolite of ethanol. It is an excellent biomarker for determining recent alcohol use and chronic alcoholism. Currently, EtG is monitored by GC-MS and LC-MS/MS methods.

METHODS: The DRI® Ethyl Glucuronide Assay is a homogeneous enzyme immunoassay with high specificity to EtG. The assay is based on the competition between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug from the urine sample for a fixed amount of antibody binding sites. Active enzyme converts NAD to NADH, resulting in an absorbance change that can be measured at 340 nm. The assay consists of two liquid ready-to-use reagents, five calibrators and two sets of controls. The assay is a dual cutoff assay using 500 ng/mL and 1000 ng/mL as cutoff calibrators.

RESULTS: The assay performance was evaluated on the Olympus AU640 analyzer in both the qualitative and semi-quantitative modes. In semi-quantitative mode, running all five calibrators 0, 100, 500, 1000, and 2000 ng/mL in calibration mode generated a standard curve. The following chemistry parameters were used:

sample volume 35 uL, reagents 1 and 2 at 80 uL each, assay method RATE 1, measuring points 13 - 17 and calibration type polygonal 5AB. Assay precision was evaluated using modified NCCLS protocol. The two cutoff calibrators and two sets of controls (± 25% from C/O calibrators) were run n=6, two runs per day for 5 days with a total of n=60 per level. The within-run and total precision for the cutoff calibrators and ± 25% controls ranged from 0.4 - 2.2% (qualitative) and 2.0 - 5.8% (semi-quantitative). The limit of detection (LOD) was 13.9 ng/mL. No significant interference was observed from endogenous substances. No cross-reactivity was observed with parent compound ethanol and glucuronides of commonly abused drugs up to 100 ug/mL. In method comparison study, 128 samples were tested in both the qualitative and semi-quantitative modes and the analysis of results by 2 x 2 tables showed > 95% concordance with the LC-MS/MS reference method. Comparison of the semi-quantitative immunoassay results with LC-MS/MS yielded the following Deming's Regression: y = 1.008 x +23.4, and correlation coefficient of 0.959.

CONCLUSIONS: The DRI Ethyl Glucuronide Assay is very sensitive, precise and convenient method for the detection of EtG in human urine. The assay can be applied to several other high throughput automated clinical chemistry analyzers.

Validation of a LC-MS/MS Method for the Determination of Ethyl Glucuronide in Human Urine and its Correlation to the DRI® Ethyl Glucuronide Enzyme Immunoassay

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AIMS: The aim of this study was to validate a LC-MS/MS method for the determination of ethyl glucuronide (EtG) in human urine and show its correlation to a homogenous enzyme immunoassay. Ethyl glucuronide is a valuable biomarker for the detection of alcohol abuse. EtG, a direct metabolite of ethanol, is water soluble and non-volatile. Ethyl glucuronide has a longer detection period than ethanol, and in chronic alcoholics it can be detected up to 5 days making it a useful tool for monitoring abstinence in withdrawal programs.

METHODS: For the LC-MS/MS method, EtG and the internal standard (EtG-D5) were extracted with a protein precipitation. All extracts were analyzed with a Micromass Quattro Micro triple quad MS that was coupled to a Waters 2795 Alliance HPLC system. The instrument was operated in a negative electrospray mode, where analysis was performed by multiple reaction monitoring m/z 221 > 75 (EtG) and m/z 226 > 75 (EtG-D5). Using a simple isocratic method, 20 µL of the supernatant was injected onto a Thermo Hypercarb column (5 µm, 2.1 mm * 100 mm) fitted with a guard column set to a flow rate of 0.325 mL/min of 5.0% acetonitrile solution containing 0.1% formic acid.

RESULTS: The calibrator range established on the LC-MS/MS was 0.1-20.0 µg/mL. The calibration curve demonstrated good linearity with a coefficient of r = 0.9989. The intra-day precision and accuracy ranged from 2.1 - 6.6% and 5.3 - 12.4%, respectively and the inter-day precision and accuracy ranged from 5.3 - 6.8% and 1.3 - 4.4%, respectively. The extraction efficiency of EtG at a concentration of 2.5 µg/mL is 94.8% and the LOD is 50 ng/mL. The DRI® Ethyl Glucuronide Assay is a homogenous enzyme immunoassay with ready to use liquid reagents, calibrators, and controls. The assay range is 0 - 2000 ng/mL. Samples used in this study were obtained from a controlled study group of 8 healthy individuals. A total of 57 urine samples were collected from the 8 individuals at different times after consumption of alcohol. The samples were tested on the Hitachi 917 analyzer using the immunoassay and the results were compared to LC-MS/MS results. The correlation of sample results using Deming's equation resulted in y = 093 x –0.6 with a correlation coefficient of 0.998.

CONCLUSIONS: The results of this study indicate that the LC-MS/MS method demonstrated good correlation with the DRI Ethyl Glucuronide assay. The LC-MS/MS method is a reliable and precise method that can be used as a confirmation method for the determination of ethyl glucuronide in human urine.

Comparison of Capillary Electrophoresis and a Direct Immunoassay (N Latex CDT) with the Traditional Method of Anion-Exchange Chromatography-Immunoturbidimetry (%CDT TIA) for the Determination of Carbohydrate-Deficient Transferrin in Alcoholised Drivers

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AIMS: Alcohol use disorders are an important issue in clinical toxicology especially for license reapplication after driving under the influence of alcohol. As patients may underestimate their drinking, laboratory tests may be important. Changes in the carbohydrate composition of serum glycoprotein such as transferrin have been reported to be superior to usual markers like gamma glutamyltranferase (GGT). Concerns about sensitivity and specificity have lead to recent refinements to analytical methods to improve performance. New analytical methods such as high performance liquid

chromatography (HPLC) or capillary electrophoresis (CE) have been developed to improve the separation and detection of transferrin isoforms. Recently, a direct immunoassay based on a monoclonal antibody that specifically measures the transferrin isoforms lacking 1 or 2 N-glycan chains (asialo-, monosialo-, and disialotransferrin) has been marketed (N Latex CDT). The assessment of these new methods in comparison with the traditional method of anion-exchange chromatography-immunoturbidimetry (%CDT TIA) need further evaluation especially in clinical practice and subjects reapplying for drivers licenses.

METHODS: We studied 245 consecutive Swiss drivers referred to the institute of legal medicine because of driving while under the influence of alcohol in 2003 and 2004. Alcohol intake was monitored by structured interviews, self-reported drinking habits, audit questionnaire as well as information provided by their family and general practitioner. Consumption is quantified in terms of standard drinks, which contain approximately 14 grams of pure alcohol. Excessive drinking has been determined by high-volume drinking: 14 or more standard drinks per week. (Ref. NIAAA: National Institute on Alcohol Abuse and Alcoholism). ROC curves were calculated for comparing sensitivity and specificity of the markers.

RESULTS: The patient demographics were as follows: n=245 (216 men and 29 women); mean age =42 years (SD:12); alcohol consumption (during the month before the CDT test): > 2 drinks per day: 80 (32.7%), ≤ 2 drinks per day: 114 (46.5%), 0 drinks per day: 51 (21%). The comparison between moderate (less or equal to 2 drinks per day) and excessive drinkers (more than 2 drinks) is shown in Table 1. The ROC Curves area for AST and ALT are not significant. The comparison between abstinent subjects (no drink the last four weeks) and excessive drinkers (more than 2 drinks per day) is shown in Table 2.

Table 1: Comparison between moderate and excessive drinkers

Marker	ROC area	95% CI	Cut-off	Sensitivity	Specificity
CDT TIA	0.64	0.57-0.72	2.6	0.76	0.39
CDT N latex	0.69	0.62-0.76	2.5	0.36	0.86
Asialo+disialo-tf	0.67	0.6-0.75	1.2	0.5	0.78
GGT	0.61	0.53-0.69	85	0.30	0.82

Table 2: Comparison between abstinent subjects and excessive drinkers

Marker	ROC area	95% CI	Cut-off	Sensitivity	Specificity
CDT TIA	0.78	0.70-0.86	2.6	0.76	0.61
CDT N latex	0.80	0.72-0.88	2.5	0.36	0.94
Asialo+disialo-tf	0.81	0.74-0.88	1.2	0.49	0.94
GGT	0.69	0.60-0.78	85	0.30	0.86

CONCLUSIONS: Capillary electrophoresis, direct immunoassay and the traditional method of anion-exchange chromatography-immunoturbidimetry performed poorly but similarly for detection of moderate alcohol use. We found also that the presence of asialo-Tf is a good predictor of non abstinence (2% of false positive) which may have strong implication for license reapplication.

Keywords: Carbohydrate deficient transferrin, Asialotransferrin, Method comparison.

Acceleration of the Average Ethanol Elimination Rate in Oral Administration Studies

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AIMS: To determine the up-to-date elimination rate per hour of ethanol in a healthy population sample considering forensic criteria.

METHODS: As part of a study on ethanol kinetics, elimination rates of blood alcohol were determined. Ninety-seven (97) sober, healthy men, aged 21 to 30 given oral alcohol in a dose of 1.10 g/Kg weight in the form of beer (4.5 Vol%), wine (12 Vol%) or vodka (40 Vol%) continuously over 2 h. All subjects were pre-examined thoroughly concerning indicative parameters of alcoholism or other liver pathology. Drinking habits showed an average weekly alcohol consumption of 130 g ethanol. Blood samples were taken in intervals of 20 minutes, starting with the beginning of alcohol consumption and ending at 0.0 mg/L breath alcohol. Therefore continuous sampling over 6 to almost 10 h was accomplished. The mean BAC of each sample was determined in quadruplicate, twice by the enzymatic ADH-method, twice by gas chromatography. Beginning 2 hours after the end of drinking linear elimination was assumed, ending with a BAC 0.2 g/Kg. In this period retrograde calculation in court (Germany) is legally allowed. The elimination rate was calculated by linear regression. The hourly elimination rate (Beta-60) was determined as the gradient of the regression line.

RESULTS AND CONCLUSIONS: For the subjects tested the following results were obtained: Mean 0.1681 g/Kg/h; SD 0.0307; Median 0.1661 g/Kg/h; Max 0.2451 g/Kg/h; Min 0.1084 g/Kg/h, mean R^2 = 0.9912 (y = a+bx). The determined hourly elimination rate beta-60 is significantly higher than the 0.15 g/Kg/h that are currently considered as normal average rate. Comparing this value with older and recent scientific work under similar circumstances, a trend towards a higher average elimination rate has to be discussed. With regard to our results a beta-60 of 0.26 g/Kg/h would have to be considered as maximum elimination rate to include 99.73% of possible cases.

A Comprehensive Analytical Protocol for Alcohol, Drugs and Traffic Safety

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AIMS: Laboratory testing in the field of alcohol, drugs, and traffic safety (ADTS) requires the mise au point of robust protocols. A protocol implemented at Forensic Toxicology and Antidoping (FTA), comprehensive of all analytical activities related to ADTS, namely driving under the influence (DUI), alcohol and drug related accidents (ADRA), driving license regranting (DLR), is described.

METHODS: Oral fluid, blood, urine, head or pubic hair, and vitreous humor are collected roadside by police, at hospitals or at FTA Unit. Screening and confirmation procedures have been implemented for drugs and psychoactive substances, alcohol, markers of alcohol use. Prior to screening/confirmation, biofluids may be pre-treated by means of liquid/liquid extraction (LLE) or solid-phase extraction (SPE). Urine adulteration is checked by measuring pH, adulterants, specific gravity and creatinine concentrations.

RESULTS: Enzyme multiplied immunoassay (EMIT) is used for psychoactive substances screening in urine; confirmation is performed either in urine or blood by GC-MS and GC-MS/MS (opiates, cocaine, amphetamines, cannabinoids, methadone) and LC-MS/MS (benzodiazepines), following SPE. Buprenorphine and LSD are screened by ELISA in urine and confirmed by LC-MS/MS either in urine or blood after LLE. For other psychoactive substances, not covered by EMIT, a systematic toxicological analysis based on LLE of neutral, basic and acidic compounds from urine or blood and GC-MS plus LC-MS/MS detection was implemented. Alcohol concentration is determined in urine and blood by headspace gas chromatography; an immunonephelometric assay is used to quantify carbohydrate deficient transferrin (CDT) in serum, followed by capillary electrophoresis – DAD confirmation of positive cases. Specific markers for recent alcohol intake are searched in serum, urine and vitreous humor (ethyl glucuronide, ethyl sulphate) by LC-MS. In hair samples, specific analytes of the classes of opiates, cocaine, amphetamines, cannabinoids are determined by GC-MS after SPE. In oral fluid, specific drugs and psychoactive substance are determined as parent drugs by LC-MS/MS after minimal sample pre-treatment.

CONCLUSIONS: The whole protocol proved to be efficient. Typical analytical times are as follows: 1 day for DUI ascertainment; 3 days for ADRA ascertainment; DLR ascertainment requires 15 days to 2 months due to multiple sample collection.

Alcoholism Biomarkers for the Assessment of DUI Recidivism

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INTRODUCTION: Assessment for alcohol use disorders (AUD) is a major objective of the evaluation process following a Driving Under the Influence (DUI) conviction. The risk of under-reporting of alcohol use in the DUI evaluation context has led to interest in the potential of biomarkers of alcohol misuse. The prevalence of AUD in this population has been found to be higher based upon biomarkers compared to self-reported diagnostic procedures. Studies using carbohydrate-deficient transferase (CDT) alone or in combination with other markers have indicated rates of AUD of 30% and 48% respectively in samples of DUI offenders. While rates of alcohol abuse or dependence have been observed to be higher in recidivists compared to first offenders when using self-report based diagnostic interviews, to our knowledge studies using biomarkers have not corroborated this relationship.

MAIN HYPOTHESES: 1) A systematic relationship exists between alcohol use biomarkers and recidivism status; 2) This relationship is strengthened when using multiple biomarkers; 3) DUI recidivists possess biomarkers indicative of greater prevalence of AUD than first offenders.

METHOD: 71 male DUI offenders (mean age = 44.2 years, mean education = 12.3 years) were included in the main analyses. The number of past convictions of DUI offenders was distributed as follow: 1 = 42.3%, 2 = 26.8%, and 3-8 = 30.9%. In addition to sociodemographic variables, blood samples were collected from participants to measure mean corpuscular volume (MCV), thiamine, alanine aminotransferase (ALT), aspartate aminotranferase (AST), gamma-glutamyl transferase (GGT), and CDT. Logarithmic transformations were used to correct the distribution of some biomarkers.

RESULTS: ALT significantly differentiated between first offenders and recidivists, $t(69) = -2.2$, $p = 0.029$. Number of DUI convictions was correlated to ALT ($r(69) = 0.25$; $p = 0.018$), AST

(r(69) = 0.21; p = 0.039), MCV (r(69) = 0.25; p = 0.019) and thiamine (r(69) = 0.22; p = 0.036). Forward stepwise regression modeling of frequency of DUI convictions was significant (R^2 = 0.14; F(2,68) = 5.35; p = 0.007) with ALT (p = 0.010) and thiamine (p = 0.018) making a significant contribution. Using established cut-offs indicative of alcohol use problems, however, the biomarkers studied individually or in combination failed to reliably differentiate recidivists from first offenders on their AUD status.

CONCLUSIONS: The study indicates a positive relationship between biomarkers of AUD and recidivism status. Furthermore, use of multiple markers improves the strength of this relationship. Nevertheless, traditional biomarker cut-offs for AUD detection may not be useful in estimating recidivism status. In contrast to detection of AUD, recalibration of biomarkers cut-offs in the assessment of recidivism may be necessary.

Epidemiology and demographics of drivers under influence (DUI)

Épidémiologie et démographie des conducteurs sous l'influence de substances psychoactives

Drink Driving and Traffic Safety in Mauritius: Magnitude and Status of Intervention

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OBJECTIVE: To provide an overview of Mauritian data on road traffic injuries associated with drink driving and also to outline the intervention programs to combat this problem.

METHOD: The main sources of data used in this study were reports and statistics released by the Central Statistics Office and the Police Authority in Mauritius. Data were also obtained from published research reports and articles.

RESULTS: Extent of the problem: Mauritius which is situated in the Indian Ocean has a surface area of 1865 km^2 and a population of about 1.2 million. Every year about 18,000 road accidents are reported to the Police. In those accidents around 150 people are killed and 250 people are seriously injured. On average 50 people are killed each year in drink drive road crashes. According to statistics available, out of a total of 1,568 alcohol tests carried out by the Police during the period January to December 2004, 485 were found to be above the prescribed limit, representing 31%. Unfortunately, the tendency has been on the increase in 2005 and 2006. Thus out of a total of 1979 interventions made during the period January to December 2005 and out of a total of 2,142 interventions made in year 2006, 767 and 858 cases were found to be above the prescribed limit, representing 38% and 40% respectively. These figures are indeed alarming. Legislation: The Road Traffic Act was amended in 2003 to reduce the prescribed limit for alcohol concentration [from 80 to 50 mg of alcohol in 100 mL of blood]. In year 2006, the Road Traffic Act was further amended to provide for the immediate suspension of the driving licence or provisional driving licence of the driver of a motor vehicle who is charged with, or reasonably suspected of causing death by careless driving whilst under the influence of intoxicating drink or driving or being in charge of a motor vehicle with alcohol concentration exceeding the prescribed limit by at least 100%.

Road Safety Intervention - Enforcement Approach: Since 1992, screening for alcohol by the roadside has made use of an Alcolyser Kit. From traditional clinical methods and blowing into balloons the Police Authority now has the Ethylometer to detect the presence of alcohol and more recently an evidential Breathalyzer equipment, namely the Portable Lion Intoxilyzer 8000 that gives printed readings of actual breath alcohol concentration. The Police Authority has also purchased a Booze Bus which allows them to conduct alcohol screenings all over the island.

Road Safety Intervention – Education Approach: To sensitize and educate drivers of the risks associated with drink driving, the Ministry of Land Transport organizes at least one hard-hitting road safety campaign every year.

CONCLUSIONS: Drunken driving is a major cause of road traffic crashes in Mauritius. Measures implemented so far as an enforcement and deterrent strategy to reduce alcohol-impaired driving include license suspension laws, alcohol sobriety testing, use of designated driver and alcohol safety education. In order to be more effective and efficient in its intervention approach, Mauritius needs to be kept abreast of experiences elsewhere.

Medicinal Drugs and Traffic Accidents

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The role of medicinal drugs in driving and traffic accidents is of increasing interest. There is limited information regarding the traffic accident risk associated with the use of potentially impairing medicinal drugs. Benzodiazepines are the therapeutic group that have

shown an increased road traffic risk. Available evidence is mainly based on linkage between data provided by police or insurance companies re traffic accidents and data re prescriptions. The aim of the study was to assess the traffic accident risk for those patients taking medicinal drugs which note, in the Summary of Product Characteristics (legal technical report according to the European Union medicinal drug regulations) and package inserts, that these medicinal drugs impair the ability to drive safely. Drivers attending two Spanish Medical Driver Test Centres for assessment of their fitness to drive were asked about the medicinal drugs they were consuming. Each registered pharmaceutical preparation was coded following the Anatomical Therapeutic Chemical classification system. Chronic and acute use was defined respectively as using the medicinal drug on a daily basis for at least one month or not. The Summary of Product Characteristics for each medication was reviewed, and whether or not there was a warning about the medication's effect on ability to drive safely was recorded. Drivers were followed for a year. Self-revealed implications for traffic accidents while taking the medicinal drugs was also recorded. The study was approved by the Ethics Committee at Valladolid's Faculty of Medicine. The study included 4,491 drivers. The SPSS 13.0 software package was used. 1,637 out of 4,491 drivers (36.5%) consumed medicinal drugs. There was a warning in the Summary of Product Characteristics concerning the effect of the medicinal drug on the ability to drive in the medication taken by 202 out of the 4,491 drivers (4.5% of drivers). Logistic regression analysis, after controlling for gender, age, mileage and alcohol consumption, showed that those taking medication with a warning in the Summary of Product Characteristics showed an increased risk of involvement in a traffic accident (OR = 2.51, 95% CI 1.63 - 3.85), while those taking a medicinal drug without such a warning did not (OR = 0.82, 95% CI 0.61 - 1.08) when compared with those drivers not taking medication (OR = 1). The present results show that 1 out of 20 drivers is taking a medicinal drug with a warning in The Summary of Product Characteristics concerning the effect of the medicinal drug on the ability to drive, and that those who take these medicinal drugs showed an increased risk of involvement in a road traffic accident as compared to those who are taking medicinal drugs without such a warning or who are not taking medicinal drugs.

This study was carried out as part of the project of the European Union IMMORTAL "Impaired Motorist, Methods of Roadside Testing and Assessment for Licensing", Contract Nº. GMA1/2000/27043 SI2.319837, program "Competitive and Sustainable Growth". This study was also supported by a grant from Redes Temáticas de Investigación Cooperativa, Red de Trastornos Adictivos, RD06/0001, Instituto de Salud Carlos III.

An Audit of Drug Use in Driver Licensing Cases

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The Drivers Medical Group of the Driver and Vehicle Licensing Agency (DVLA) processes around 500,000 cases a year from individuals in whom there is at least preliminary evidence that they may have a medical condition that could affect their fitness to drive. A proportion of these require independent medical examination and assessment, and in some cases assessment for drug misuse or drug dependency. Approximately 66% of the drug cases referred for Laboratory analysis are from High Risk Offenders (HRO's), with a further 8% referred due to a police notification. An audit of the cases submitted to the Regional Laboratory for Toxicology, City Hospital, Birmingham, UK by the DVLA for drugs of abuse analysis was conducted to determine the prevalence of amphetamines, benzodiazepines, cannabinoids, cocaine, ecstasy, methadone, opiates and propoxyphene. A total of 9,893 urine specimens were received in the Laboratory between July 2002 and December 2006, representing 8,076 cases collected from subjects ranging between 16 and 75 years of age. With the exception of propoxyphene, all specimens were initially screened for the drugs of interest using either an Olympus AU600 or Olympus AU640 analyser in conjunction with CEDIA® reagents according to manufacturer's instructions. Propoxyphene screening was performed using an in-house GC-NPD method. All positive results were confirmed according to the UK Workplace Drug Testing guidelines using fully validated GC-MS methods. Of the total number of specimens received, 191 (2%) were rejected for analysis and not processed, 3,368 (34%) were positive for one or more of the drugs of interest and a further 6,334 (64%) were found to be negative. Of the negative specimens 11% were identified as dilute, i.e. the creatinine concentration was less than 1.8 mmol/L, and a further 1% were found to have pH readings of either less than 4 or greater than 9.The cannabis metabolite, 11 nor-9-carboxy Δ^9-tetrahydrocannabinol (THC-COOH) was found to be the most prevalent analyte being detected in 54% of positive cases. Other frequently detected analytes included opiates (16.5%), benzodiazepines (16%) and methadone (15.7%). Poly drug use was determined in a total of 12% of the specimens analysed.

The data presented in this poster provides an insight in to the prevalence of drug use in this population. A summary of the concentration ranges determined for the drugs of interest together with a review of the number of repeat analyses and positive cases due to declared medication will be presented.

Distribution of Abused Drugs in 275 Alcohol-Positive Blood Samples of Korean Driver

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BACKGROUND: Even though driving under the influence of drug (DUID) is a worldwide problem, we, Korea, have no regulation system yet except for alcohol, and there are little cases reported related to DUID. In order to investigate the type of abused drugs for drivers in Korea, we tried to analyze controlled and non-controlled drugs in alcohol-positive blood samples.

METHODS: 275 whole bloods, which were positive for alcohol on the roadside test, were collected from the police for two months (November ~ December 2006). The analytical strategy was constituted of three steps: First, alcohol in blood samples were confirmed and quantified by gas chromatography. Second, controlled drugs were screened by evidence investigator™ (Randox, U.K.) as preliminary test. It was based on competitive enzyme immunoassay by biochip array analyzer. Eight groups of drug abuse were screened: amphetamines, cannabis, cocaine, opiates, barbiturates, methadone, benzodiazepines group I (as oxazepam) and benzodiazepines group II (as lorazepam). Finally, confirmation of these drugs was performed by GC-MS. Blood samples were extracted by solid-phase extraction by RapidTrace™ (Zymark, U.S.A.). After trimethylsilyl (TMS) derivatization, eluates were analyzed to GC-MS. Total 51 drugs were investigated in this study including controlled drugs, antidepressants, 1st generation antihistamines, dextromethorphan, nalbuphine, ketamine, etc. For rapid detection, we developed the automated identification system, by registering both their retention times and mass spectra as the standard library data.

RESULTS: Concentrations of alcohol in 275 blood samples were ranged from 0.011 to 0.249% (average, 0.119%). Among 149 blood samples, just ten samples (6.7%) showed positive results to the immunoassay: three amphetamines, one cannabis and six benzodiazepines group I. Besides controlled drugs, confirmation for other drugs related to DUID are in process by GC-MS. Even though the frequency of drug abuse for Korean drivers was relatively low in this study, these results were limited to alcohol positive blood samples, so it is necessary to analyze more samples including alcohol negative blood.

Alcohol and Drugs in Suspected Impaired Drivers in Ontario from 2001 to 2005

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Breath samples are the most frequently obtained sample for determining blood alcohol concentrations (BAC) in suspected impaired drivers in Canada; however, when a peace officer has reasonable and probable grounds to believe that due to the physical condition of a driver it is either impractical to obtain a breath sample or that the driver is incapable of providing a breath sample, then the peace officer can make a demand for blood. This study retrospectively examines drug and alcohol findings in blood samples collected from suspected drinking drivers over a five-year period.

There were 733 suspected impaired driving cases submitted to our laboratories for the purpose of blood alcohol testing during the study period. Males represented the largest percentage (n = 623, 85%) and ranged in age from 16 to 83 years old (mean = 36). Female drivers (n = 110) ranged in age from 15 to 72 years old (mean = 35). BACs, measured by headspace gas chromatography–flame ionization detection, ranged from not detected (ND) to 414 mg/dL (mean = 165) for males and ND to 425 mg/dL (mean = 160) for females. Alcohol was detected in 708 cases, with the majority of cases (n = 640, 90.3%) having a concentration of 80 mg/dL and greater at the time of sampling. The majority of drivers were involved in a motor vehicle accident (MVA; n = 658, 89.8%), with single MVAs (n = 412, 56.2%) being most common. The language of the Criminal Code of Canada states that the purpose of a blood demand is to "enable proper analysis to be made in order to determine the concentration, if any, of alcohol in a person's blood", however, it is the practice of this laboratory that when an analysis yields a BAC of less than 100 mg/dL and/or there is reported history of specific or suspected drug use, then analysis for drugs are generally performed. In this study, 16 cases (2%) were analyzed for alcohol and one other specified drug, and in 26 cases (4%), more extensive analyses were performed, that is general screening for pharmaceuticals and drugs of abuse. Of the aforementioned cases, 34 had positive drug findings. The drugs detected most frequently were: Δ^9-THC (n = 18; <1 - 10 ng/mL), benzoylecgonine (n = 8; 0.13 - 6.3 mg/L), and cocaine (n = 6; < 0.13 - 0.29 mg/L), morphine (n = 6; <15 - 101 ng/mL), lorazepam (n = 5; 10 - 501 ng/mL) and diphenhydramine (n = 4; < 0.13 mg/L). The findings reported herein represent an examination of blood samples from impaired drivers in Ontario from 2001 to 2005 where a demand for blood was made by a peace officer. Of 733 drivers, 97% were alcohol positive, and of these, 90% had a BAC ≥ 80 mg/dL, the legal limit in Canada. Analyses for drugs

other than alcohol performed in a small subset of these drivers demonstrated that THC, cocaine, and morphine were the most frequently encountered drugs. The data reported above is reflective of drug use in those drivers where such analyses were performed; however, it cannot be used to determine the frequency of drug use by all drivers in Ontario. The data demonstrates that "drug-driving" does occur and that there is a need for a systematic and comprehensive investigation of drug use in drivers in Ontario.

Intoxication Assault, Intoxication Manslaughter: Is Drug Analysis Necessary or Can the Alcohol Result Stand Alone?

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The state of Texas has the driving offences of intoxication assault (IA) and intoxication manslaughter (IM). Current laboratory policy requires that a complete drug analysis be performed regardless of the blood alcohol concentration. The policy for a routine DWI is that a drug analysis be performed only when the blood alcohol is ≤ 0.08. Should this policy be employed for IA and IM? What follows is a ten year retrospective of drug analysis results for IA and IM offences with blood alcohol results of ≥ 0.09 for cases in Dallas, Texas. Within these ten years, there were ~15,000 suspected DWI/DUID blood cases, ~450 were IA or IM. Of these IA and IM cases 211 had blood alcohol results of ≥ 0.09. The 47 drug positive cases contained drugs in the range of substances of abuse, prescription medications, and hospital drugs. Some drug levels could be considered contributory to impairment, however, the major effect is thought to be in prejudicing a jury.

Alcohol, Drugs and Driving in 21. Century Iceland

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AIM: To take a retrospective look at results from blood samples from Icelandic drivers measured for alcohol and drugs in our laboratory from 2001 to 2006.

MATERIAL AND METHODS: The study material includes all blood samples submitted by police authorities to our laboratory for analysis of alcohol and drugs over the last 6 years. The total number of samples was 12,270. Alcohol was measured in all samples and drugs were analysed in 568 (4.6%). Drug analysis was done as reqested by the police and in some cases that limits the number of drugs reported.

RESULTS: 10,362 (84.5%) of samples were over the legal limit for blood alcohol (0.5%). The results show a marked increase in the number of samples submitted for drug analysis, specially in the years 2004-2006. A majority of the samples analysed for drugs were under the legal limit for alcohol. More than one drug was analysed in many cases and on average there are reported 1,58 drugs per blood sample. Over the study period the most common drugs analysed were benzodiazepines (285 or 50%), amphetamines (272 or 48%) and tetrahydrocannabinol (153 or 27%). In 2006 there were 114 (60%) samples positive for amphetamines, 66 (35%) samples positive for benzodiazepines and 54 (29%) positive for THC.

DISCUSSION: In Iceland now there are 1.5 inhabitants per car. Statistics for traffic deaths in 2006 show that driving under the influence of alcohol was found to be a cause in 25% of the cases. Driving under the influence of drugs seems to be an expanding problem in Iceland. In 2006 a zero tolerance limit for illicit substances in blood and/or urine was introduced in Icelandic traffic law. Our results indicate that the abuse population in Iceland uses a mixture of illegal substances and prescription drugs. Last years increase in the number of drivers positive for amphetamines in blood fits with an increase in amphetamine abuse reported by drug treatment facilities in Iceland. Amphetamine was also the most common illegal substance seized by police and customs last year.

CONCLUSIONS: The number of cases of suspected drivers under the influence of alcohol and/or drugs is of major concern. The effects of the recent change in Icleandic traffic law regarding illegal substances and driving on this problem will be seen in the near future.

Rural and Urban Differences in Kentucky DUI Offenders

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Recent national data suggests that the prevalence of DUI is higher in rural areas than in more urbanized areas of the United States. Yet, little is known about rural DUI offenders, particularly those living in very remote areas. This is a significant gap in the DUI literature in that rural areas have been shown to be increasingly less protected from alcohol and drug problems. Many rural areas have been shown consistently to have high rates of alcohol and drug use, abuse and dependence coupled with high unmet substance abuse treatment need. Despite this documented substance abuse problem in rural America, few studies have examined rural DUI offenders or compared them to DUI offenders from urban areas. The present study examined a total of 21,025 substance abuse assessment records for persons convicted of DUI in Kentucky and who concluded treatment in 2005. Assessment records included demographic characteristics, AUDIT scores, DAST scores, and the DSM-IV substance use

disorder checklist. Beale codes were used to define the extent to which the county of conviction was rural (1 = urban, 9 = very rural) and records were then categorized based on this code. Data were analyzed using correlational and chi-square analysis. Positive, and statistically significant, associations were found between rurality and DAST scores, DSM-IV substance abuse and dependence disorders, and rates of treatment non-compliance. Interestingly, only AUDIT scores and blood alcohol concentration were negatively associated with rurality. The study suggests that problem severity among DUI offenders may be greater in rural areas, particularly with drug use given their elevated DAST scores. The availability of substance abuse treatment, however, is scarce in many rural areas. As a result, practitioners may face greater challenges in assessing and providing appropriate treatment for this group of DUI offenders which may put them at greater risk for continued impaired driving.

Pharmacoepidemiological Studies of Medication Use and Crash Risk: Available Data Sources - Opportunities, Limitations and Future Challenges for Road Safety Research

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While the relationship between alcohol use and crash risk is well recognised, the impact of other drugs on road safety is less clear. This is especially so for prescription medications. An overwhelming methodological concern for epidemiological studies of the relationship between medication use and crash risk is the huge sample size required to detect any relationship, considering that both the outcome (traffic crashes) and exposure (prescription medication use) are rare. Sample size requirements are compounded when investigators wish to look beyond the simple relationship between a particular drug class and crash risk, in order to focus on the risk associated with specific medications and the effect of dosage and duration of use. Yet this is essential if such research is to effectively inform the prescribing decisions of health practitioners who are being encouraged to prescribe the least impairing drugs. The most efficient and feasible method of obtaining such large sample sizes is to use pre-existing data sources, for example, linked health databases. These bring together information on encounters with the health care system for individuals within a population, including information on dispensed prescription medications and involvement in injurious traffic crashes. They provide an exciting opportunity to study associations between rare exposures and rare outcomes. Several regions around the world that have linked (or linkable) health databases that can be used for population based pharmacoepidemiological investigations. This paper will outline an epidemiological PhD research program aimed at estimating crash risk associated with medication use. The information necessary to conduct large-scale pharmacoepidemiological investigations of the association between medication use and crash risk will be identified, including consideration of potential confounders. The internationally available data sources will be identified and the results of a feasibility study that assessed these data sources in terms of the information requirements will be presented. Limitations, in particular, information that is not currently available through linked health databases, will be highlighted. Finally, the role of road safety professionals in advocating to promote augmentation of linked health databases with information from other sources to better address issues in road safety research will be discussed.

The European Project DRUID - Scientific Support for Combating Impaired Driving

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The objective of DRUID (Driving Under Influence of Drugs, alcohol and medicines) is to give scientific support to the EU transport policy to reach the road safety target of reducing the number of killed road users in the EU by 50% between 2001 and 2010 by establishing guidelines and measures to combat impaired driving. DRUID consists of seven Work Packages:

WP0 "Management" embraces a number of governance tasks: overall co-ordination and scientific management; financial management; quality assurance; liaison between the Commission, the Steering Committee and partners; development and implementation of Consortium Agreement; representation of the Consortium; management of intellectual property rights; organisation of dissemination activities; reporting, etc.

WP1 "Methodology and research": The aim of WP1 is to establish a theoretical framework and an integrative methodology. Different experimental studies with psychoactive substances will be conducted to assess driving performance and estimate the risk for different substances and to formulate recommendations for thresholds.

WP2 "Epidemiology": The objectives of this work package is to assess the situation in Europe regarding the prevalence of alcohol and other psychoactive substances in drivers in the general traffic and drivers involved in injury accidents, to calculate the accident risk for drug impaired drivers and to identify characteristics of drug impaired drivers.

WP3 "Enforcement": The objective of WP3 is to evaluate roadside testing devices both from a scientific perspective and an operational police perspective. A cost-benefit analysis will be carried out to find out which on-site screening device will have the best cost-benefit rate and to what extend enforcement of driving under the influence of psychoactive substances is cost beneficial for society.
WP4 "Classification": This WP reviews the existing classification and labelling systems regarding medical drugs and driving and a proposal will be made for the criteria and the methodology of a European system. Furthermore, a methodology will be developed to update this system continuously.
WP5 "Rehabilitation": The overall aim of work package 5 is to increase knowledge regarding rehabilitation of drivers with drunk-driving or drug-driving offences. The research will provide fundamentals for establishing adequate and effective rehabilitation measures throughout Member States according to uniform defined criteria and quality standards.
WP6 "Withdrawal": The objectives of this work package are to review the state-of-the-art , collect and evaluate practices in various European countries based on the former related studies, assessment of the effect of various strategies regarding withdrawal of driving licence with focus on the conditional driving license withdrawal and development of recommendations with a comprehensive view on the entire problem.
WP7 "Guidelines and dissemination": The objectives of this work package are to review the state-of-the-art and documented effectiveness of existing campaigns and practice guidelines regarding psychoactive substances, focussed on the general public and health care professionals, development of information materials for general public and health care professionals and developing proposals for improving procedures for assessing fitness to drive.

Roles of Ethnicity in the Age of Drinking Initiation in Nigeria

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Nigeria is a multi ethnic, multi tribal and multi cultural land. There is no ethnic group in which alcohol use does not exist. However alcohol use is so well documented in some ethnic groups such that it warrants special reference. The Ogoni, Ikwere, Kalabari, Ijaw, Itsekiri, Urhobo, Ika, Ejagam, Yala, Efik, Bini in the Niger Delta, the Igbo in the East, the Tiv and the Birom in Benue Plateau and the Kaje in the North of Nigeria. In some cultures alcohol is a necessity in the practice and performance of most of the traditional rites. Alcohol is involved in the rites of marriage, rites of passage, child birth and naming ceremonies, marriage, graduation, birthday, and burial ceremonies. Infants, children and adolescents get exposed and initiated into the use of alcohol at different stages depending on ethnic or tribal background. Gin brewing depots are common place along river banks, a business which is dominated by the Urhobo, Itsekiris and the Ijaw ethnic tribesmen. This product is distributed through a wide network, and readily finds itself on the tables of most households. It is the traditional African gift from the elders that symbolizes the 'cherished' African hospitality Abudu community in Orhionmwon local government Area is comprised of people from more than 15 ethnic backgrounds. These people have been living together harmoniously for over a century in spite of their cultural differences. Their traditions are strictly protected and jealously guided. This is a study of the influence of ethnicity on the age of drinking initiation in Nigeria.

METHOD: Six major ethnic groups living in Orhionmwon community of Edo state were selected for this study. A sample of 10 households from each of the 6 tribes was used. The heads of household were interviewed using a common questionnaire. The issues raised in the questionnaire include age of first contact with alcohol, age of exposure to alcohol, circumstance, nature of drink used, sex and ethnicity.

RESULTS: Average age of first contact with alcohol was 5½ months. Average age of secret gang-drinking was 11 years. Average age of tolerated open drinking was 16 years. Average age of full involvement in alcohol was 25 years. Both sexes are affected. All the traditional rites are performed with the use of the illicit gin. Alcoholic drinks are used freely for entertainment during all ceremonies.

CONCLUSIONS: Ethnicity plays a major role in the early-age formation of alcohol drinking habits.

Alcohol Use Among Junior Secondary School Students in Nigeria

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Alcohol use is the most fancied method chosen by students to display attainment of adulthood among youths. Many students use alcohol and cigarettes to establish status symbol and attract the attention of colleagues especially the female students. This behaviour emanates from a society that seems helpless in the face of value decay that has engulfed the African traditional institution in the name of globalization. The use of weeds, alcohol and other drugs is rampant in most communities in Nigeria today. The female students are not spared by this virus. Most students pick up the habits in schools. This follows the unrestricted sale of alcohol and other substance of abuse in staff quarters. Outside school, students join a community of adults who consume drugs freely recklessly and wantonly. The consequence of this is that student form gangs whose sole objective

is to use and share drugs and learn various shades of terrorist acts.
The purpose of this study is to determine the rate of alcohol use among adolescent junior secondary school students in a Local Government Area in Nigeria.

METHODS: Questionnaires were administered to 80 randomly selected students in year 1-3 drawn from 4 secondary schools. The questions were crafted in such a way that it did not give away the purpose. Issues in current affairs, religion, social studies were mixed with a few others targeting drug and alcohol use. The questionnaires were administered one on one but allowing freedom for the subjects.

RESULTS: 40% of the students have used alcohol at least once in their life. 26% drank an alcoholic beverage to the point of intoxication, 32% had used their own money to buy an alcoholic drink for personal consumption in the past while 48% never had any contact with alcohol.

CONCLUSIONS: Alcohol is consumed by a large number of secondary school students in rural Nigeria.

Alcohol Use Among Subjects Who Drink on Premises of Gas Stations of Porto Alegre, Brazil

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BACKGROUND: Drinking on the premises of "gas stations" with co-located convenience stores is common among youth in Brazil. Subjects purchase alcohol at the store, drink with others on the premises, and then drive away. A city law was passed in 2006 which prohibited alcohol use on such locations but did not prevent sales on stores.

OBJECTIVES: To compare risk behaviors for traffic accidents and BAC among youth who drank alcohol on the premises of "gas stations" before and after a city law was passed.

METHOD: Interviewers purposively selected gas stations before (Time I) and after (Time II) the implementation of the law. Time I (n = 62) and Time II data (n = 50) were compared. Data were collected in two weekends before the law was passed and in two weekends one month after the law was passed. Inclusion criteria were: 1) 15 years or more; 2) drinking alcohol on the spot; and 3) car driver/passenger. Substance use and traffic risk behaviors were collected using a self-administered questionnaire. BAC was estimated by breathalyzer.

RESULTS: 73 subjects were approached and eleven (13.7%) refused to participate. At Time II, 54 subjects were approached and four refused (9.3%). They were similar on demographics and risk behaviors for traffic accidents. Mean ages were 22.7 (+/- 5.0) and 22.5(+/-4.1) and mean years of education were 13.43 (+/- 3.0 years) and 13.1(+/-2.4 years). BAC over 0.06% was found in 36% of subjects at Time I and 40% of subjects at Time II ($p = 0.62$). 9.7% of the Time I group and 16% of the Time II group with BAC > 0.06 mg/dL reported they would drive in the next two hours ($p = 0.38$). Self reported current marijuana use was also high at 12.9% and 12% respectively.

CONCLUSIONS: Over one-third of subjects had BACs over the legal limit and intended to drive soon, and alcohol use level did not change after a city law passed. Such study is feasible with low refusal rates, and may help understand the enforcement of alcohol availability laws, as well as to decrease drinking and driving among youth in developing countries.

Presence of Ethanol and Illegal and Psychoactive Drugs in Drivers Killed in Road Accidents in Southern Spain from 2004-2005

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Death due to road accidents is one of the main causes of death in Spain. This study presents the results of blood samples obtained from drivers killed in road accidents in the South of Spain during a two-year period (2004-2005). During this period, the Spanish National Institute Toxicology, Seville, received 952 blood specimens from fatal road accidents, being men involved in 83.33% of them, whereas women were only involved in 15.75%. People under 40 years old were most frequently (55.76%) involved in accidents.Toxicological analyses were performed in all samples received following our laboratory normal procedures. Ethanol was analysed by means of headspace GC-FID. Screening of drugs of abuse was performed by means of homogeneous enzyme immunoassay CEDIAÒ. Then SPE (Bond-Elut, certified) was performed in all the samples and the extracts were analysed by gas chromatography with NPD, high performance liquid chromatography (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). Ethanol was detected in 427 (44.85%) cases. Blood ethanol concentration (BAC) ranged from 0.1 to 4.11 g/L. In 135 (31.61%) of these cases others drugs were also detected. Drivers were classified in two groups, those with BAC under 0.5 g/L, (legally admitted BAC for car drivers in Spain) and those with BAC over 0.5 g/L. In a 53.63% in cases with only ethanol detected and a 68.62% in cases with both ethanol and drugs, BAC was over 0.5g/L. With respect to the drugs, the most commonly detected drugs were cocaine compounds

(n = 44), benzodiazepines (n = 37), cannabinoids (n = 14), methadone (n = 8), and opiates (n = 2). Among these 135 cases, benzoylecgonine was found in 31.85% (from 0.05 to 38.75 mg/L, median 2.62 mg/L), midazolam in 18.5% (from 0.01 to 0.22 mg/L, median 0.06 mg/L), cocaine in 14.07% (from 0.02 to 0.27 mg/L, median 0.08 mg/L), 11-carboxy-Δ^9THC-COOH was found in 10.37% (from 6.3 to 33.5 ng/mL, median 19.16 ng/mL), Δ^9-THC in 10.37% (from 2.1 to 73.3 ng/mL, median 17.41 ng/mL), nordiazepam in 8.88% (from 0.01 to 0.93 mg/L, median 0.38 mg/L), methadone in 5.92% (from 0.01 to 0.5 mg/L, median 0.16 mg/L), and diazepam in 5.18% (from 0.02 - 0.35 mg/L, median 0.14 mg/L). Other pyschoactive drugs were found in less than 1% of the cases.

From the results, we can conclude that ethanol is still the most frequent drug involved in road accidents in our area. Approximately half of the cases had BAC over the legal admitted limit for drivers, being most of them men under 40 years. However, it is noteworthy to point out the incidence of positive cases for cocaine and cannabinoids reflecting the actual situation of drug consumption in Spain.

Alcohol, Illicit and Medicinal Drugs Involved in Fatal Accidents in the North West of Spain

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The results of epidemiological studies and statistics indicate that the trend of drug use and drug abuse is increasing among drivers in Spain. In our country male drivers up to the age 22 are more than twice as likely to be involved in automobile accidents compared to their percentage of the driving population. One of the major goals in traffic safety is, to prevent or reduce the risk of causing accidents, by this group of drivers. Use of alcohol and illegal drugs by young drivers are the most important additional causes of accidents. It will be interesting to observe the development of traffic safety among young male drivers relating it with rules and laws developed in different countries to punish the influence (Zero tolerance, value limits).

MATERIAL AND METHODS: In total 253 deceased drivers were undergoing a forensic autopsy. Femoral blood was sent to the Forensic Toxicology Service of the Institute of Legal Medicine for a determination of the blood-alcohol concentration (BAC). In order to study the epidemiology of illicit and medicinal drug use, we selected these samples to analyze the presence of the most relevant substances with standard methods in use at the laboratory. In order to analyze these samples for cannabinoids, opiates, cocaine, amphetamines, benzodiazepines and antidepressants, validated LC-MS/MS technique was used after solid phase extraction.

RESULTS: Of the samples analyzed between 2004 and 2006, 76% represented males and 24% females between 19 and 74 years. Cocaine, cannabis and amphetamines were, in this order, the illicit drugs most frequently detected. Among medicinal drugs, benzodiazepines were the medications most often used. Alcohol was found in 43%. Of the samples analyzed, 84% represented males and 16% females (23 - 64 years). The alcohol levels varied from 0.1 to 3.2 g/L. The detected levels of drugs were correlated with the degree of impairment. The frequencies for multidrug use were very high. The frequencies for combined use of drugs and alcohol were 89%. Only 3.4% of drivers with positive drug findings were women.

CONCLUSIONS: In more than a third of drivers deceased in traffic accidents in Spain, alcohol and/or drugs (illegal and legal) were found. Alcohol was the commonest finding. The data presented reveal that licit and illicit substances are regularly found in drivers or victims of road accidents in Spain.

Forensic Alcohol Findings in Cases of Alleged Driving Under the Influence of Alcohol in the City and County of San Francisco, California over the 5-Year Period: 2002-2006

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This paper outlines the forensic alcohol results from 2,313 cases of alleged driving under the influence of alcohol (DUIA) analyzed at the Forensic Laboratory Division of the Office of the Chief Medical Examiner of the City and County of San Francisco between January 1, 2002 and December 31, 2006. This laboratory is one of 42 local forensic alcohol laboratories in California that is authorized to conduct chemical testing of blood and urine in accordance to California's Code of Regulations (Title 17, Div. 1, Ch. 2, Subch. 1, Gr. 8: Forensic Alcohol Analysis and Breath Alcohol Analysis, Art. 1-8, Sec. 1215-1222). In accordance to Title 17's provisions, the laboratory analyzed case specimens comprising of either whole blood or urine for ethyl alcohol. Alcohol or other volatile organic disinfectants were not used to clean the skin where a blood specimen was collected by venipuncture. Instead, aqueous benzalkonium chloride was used. The blood was always mixed with an anticoagulant and a preservative. Approved urine specimens for forensic alcohol determinations were collected no sooner than twenty minutes after first voiding the bladder into containers containing a preservative. Duplicate analyses of blood or urine specimens were performed by headspace gas chromatography with flame ionization detection (HS/

GC/MS). The results were interpreted with respect to the level of ethyl alcohol detected and an attempt was made to distinguish any epidemiologic trends over the 5-year period. Ethyl alcohol was detected in 2,242 of all cases (97%). It was detected at concentrations of 0.05% (w/v) or greater in 2,208 of all cases (95%) and in concentrations of 0.08% (w/v) or greater in 2,083 of all cases (90%). In 2002, there were 64 female and 381 male drivers arrested for allegedly violating California's DUIA laws with ethyl alcohol concentrations ranging in females from 0.00% (w/v) to 0.43% (w/v) with a mean value of 0.16% (w/v) and in males from 0.00% (w/v) to 0.43% (w/v) with a mean value of 0.17% (w/v). In 2003, there were 63 female and 382 male drivers arrested for allegedly violating California's DUIA laws with ethyl alcohol concentrations ranging in females from 0.00% (w/v) to 0.40% (w/v) with a mean value of 0.17% (w/v) and in males from 0.00% (w/v) to 0.39% (w/v) with a mean value of 0.16% (w/v). In 2004, there were 94 female and 429 male drivers arrested for allegedly violating California's DUIA laws with ethyl alcohol concentrations ranging in females from 0.00% (w/v) to 0.40% (w/v) with a mean value of 0.17% (w/v) and in males from 0.00% (w/v) to 0.40% (w/v) with a mean value of 0.16% (w/v). In 2005, there were 70 female and 416 male drivers arrested for allegedly violating California's DUIA laws with ethyl alcohol concentrations ranging in females from 0.00% (w/v) to 0.38% (w/v) with a mean value of 0.18% (w/v) and in males from 0.00% (w/v) to 0.37% (w/v) with a mean value of 0.16% (w/v). In 2006, there were 66 female and 348 male drivers arrested for allegedly violating California's DUIA laws with ethyl alcohol concentrations ranging in females from 0.06% (w/v) to 0.35% (w/v) with a mean value of 0.17% (w/v) and in males from 0.00% (w/v) to 0.39% (w/v) with a mean value of 0.16% (w/v). Using US Census age groupings, 64% of the women and 58% of the men arrested in this 5-year period belonged to the young adult group (18 to 34 years), 31% of the women and 36% of the men belonged to the middle age group (35 to 54 years) and 5% of both women and men belonged to the older adult group (55 to greater than 75 years). Focusing on the time of arrest during this 5-year period, 63% of both females and males were arrested between 01:00 hrs and 05:00 hrs, 18% of females and 19% of males were arrested between 21:00 hrs and 01:00 hrs and 10% of both females and males were arrested between 05:00 hrs and 09:00 hrs.

The findings over this 5-year period as described in this paper serve as a useful resource to the toxicologic and medico-legal communities regarding the extent of the DUIA problem in San Francisco. From the data presented herein, it appears that law enforcement agents in this jurisdiction may not be adequately resourced or adequately trained in the recognition and interception of drivers with relatively lower but still impairing levels of ethyl alcohol in their blood stream. It is also surprising that, on average, females appear to consistently have higher ethyl alcohol concentrations in their blood stream than males given that much fewer females get arrested by law enforcement agents for alleged DUIA offences in this jurisdiction and that San Francisco, unlike the rest of California and the USA, according to the US Census has more male than female residents (51% versus 49%).

A Demographic Study of Blood and Breath Alcohol Findings from Drivers Allegedly Driving Under the Influence in San Francisco, California from 2002 to 2006

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San Francisco is a dynamic city with a diverse populace of 739,000. Notably, the daytime population swells to nearly 1 million due to the employment the city offers. Between January 1, 2002 and December 31, 2006, 9, 174 DUI investigations were undertaken in the City and County of San Francisco for which blood alcohol values were obtained. Historically in this jurisdiction, direct blood alcohol analysis is performed in duplicate by headspace gas chromatography with flame ionization detection (HS/GC/FID) by the Toxicologists of the Office of the Chief Medical Examiner, while breath-derived blood alcohol values are measured in duplicate by trained law enforcement personnel with Intoxilyzer® 5000s (CMI) which are calibrated and serviced by the San Francisco Police Department's Criminalists. All testing takes place in accordance with California's Code of Regulations (Title 17, Div. 1, Ch. 2, Subch. 1, Gr. 8: Forensic Alcohol Analysis and Breath Alcohol Analysis, Art. 1-8, Sec. 1215-1222). This work is aimed at comparing blood and breath-derived blood alcohol concentrations, finding similarities or differences and determining the root cause of any.

In each of the years from 2002-2006, men represented a significant majority of the DUI investigation population, from a high of 83% in 2002 to a low of 80% in 2005. The number of breath tests administered over the five year period dropped significantly, about 20%, from 1,599 tests in 2002, 1,358 tests in 2003, 1,326 tests in 2004, 1,295 tests in 2005 down to 1,283 tests in 2006. The number of blood tests administered did not exhibit a similar trend (445; 445; 523; 486; 414, respectively). Average breath tests values for men and women were fairly constant over time; remarkably men and women had the same average for each of the five years (0.13, 0.13, 0.12, 0.12 and 0.13% w/v, respectively). This is not true of the HS/GC/FID analyzed blood alcohol data. Not only were the average values higher for the blood analysis results than for the breath-derived population,

the average for females in the blood analyzed population almost always gave higher results than the blood alcohol average for the males (female average/ male average; 0.16/0.17; 0.17/0.16; 0.17/0.16; 0.18/0.16; 0.17/0.16, respectively). With some exceptions for equipment failures, the law in San Francisco permits the subject to select either blood or breath testing. Males opted for a blood draw a higher percentage of the time (22%, 26%, 28%, 29% and 25% from 2002-2006, respectively) than did females (19%, 19%, 28%, 19% and 20%, respectively). The average age of the male subjects varied little over this five year study (34, 34, 33, 33, 33 years old, respectively); likewise the average age of females was fairly constant, though slightly younger (32, 32, 30, 31, 31 years old respectively). The majority of all DUI investigations took place between the hours of 0100 and 0500 for both breath and blood. As a crime statistic, there are 5 DUI investigations for every 2,000 residents on an annual basis.

The findings in this work are important both as an affirmation of what has already been well-established in the literature (for instance the continuing observation that average breath alcohol-derived blood alcohol concentrations are statistically significantly lower than blood-derived blood alcohol concentrations, ranging from 0.03 to 0.06 in this study), as well as serving to contrast with findings in other jurisdictions (as an example, it was reported that a higher percentage of women opted for blood than breath measurements elsewhere, while in San Francisco, a higher percentage of men than women opted to provide a blood specimen for analysis). Importantly, in San Francisco it was found that for each of the five years included, the average blood alcohol level for the male or female breath-analyzed driver was over 1.5 times the legal limit of alcohol in the United States (0.08% w/v), whereas the average directly-measured blood alcohol level for either sex was over 2 times the legal limit. While these results are an important element in the characterization of the DUIA problem in San Francisco, these data when combined with information from other metropolitan areas, could be used to illustrate the need for law enforcement to recognize the continuous issues surrounding DUIs and the magnitude of impaired driving in order to devote appropriate resources accordingly.

Use of Drugs of Abuse and Alcohol in Less than 30-year-old Drivers Killed in a Road Crash in France. Cannabis and Alcohol Shoulder to Shoulder

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OBJECTIVE: On February 2003, a law passed in France that punishes driving under influence of drugs of abuse with a zero-tolerance limit together with a penalty of up to € 4500. The aim of this study was to determine the prevalence of cannabinoids, opiates, cocaine metabolites, amphetamines and alcohol in blood samples obtained from drivers killed in road accidents in France in 2005 and 2006.

METHODS: Sixteen French toxicology laboratories participated in the study. All these laboratories satisfy an annual external quality control program. In the protocol, we included blood samples provided by law enforcement officers, from less than 30-year-old drivers killed in a road accident. Drugs of abuse and ethanol were analysed by GC-MS and GC-FID, respectively.

RESULTS: 951 blood samples were included in this study. The results are listed below:

Compound (positivity threshold)	Number of determinations	Positive cases	Prevalence (%)
THC-COOH (> 2 ng/mL)	945	330	34.9
THC (> 0.5 ng/mL)	945	259	27.4
Morphine (> 20 ng/mL)	932	24	2.6
Amphetamines (> 20 ng/mL)	928	10	1.1
Benzoylecgonine (> 20 ng/mL)	932	24	2.6
Ethanol (> 0.1 g/L)	585	216	36.9
Ethanol (> 0.5 g/L)	585	184	31.4

The highest prevalences were observed for cannabis and alcohol. 34.9% of drivers had consumed cannabis as documented by the presence of THC-COOH in blood. 27.4% of drivers could be considered under influence of cannabis at the moment of the accident because THC, the most active of the principle constituents in marijuana, was detected in blood. Among drivers positive for cannabis, THC was detected as a single drug of abuse in 92.7%, associated with cocaine, amphetamines, opiates and alcohol in 5.0, 1.6, 1.4 and 26.5% respectively. In France the tolerance-limit for alcohol is 0.5 g/L in opposite to the zero-tolerance limit for drugs of abuse. Therefore, in our study, the highest prevalence of drivers committing an offence was observed for cannabis use.

CONCLUSIONS: A previous epidemiological study performed in France had reported a lower prevalence of cannabis use (8.8%) among drivers involved in a road crash. The reasons of such a difference may be because in our study we included only less than 30-year-old drivers and above all because our protocol study concerned only killed drivers, avoiding the problem of time delay between the moment of accident and blood sampling. Our results demonstrate that the efforts of information and roadside testing should be as important for drugs of abuse as for alcohol.

Young Adult Driving After Using Drugs

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The objectives of this paper were to understand 1) the extent of self-reported drug-driving among a population of young adults, 2) the relationships of drug-driving to offense outcomes in traffic data, and 3) the characteristics of those who report drug-driving. A longitudinal study

of high-risk drinking and drink-driving surveyed by telephone the substance use, driving behaviors, and psychosocial characteristics of 5,464 young adults. The averaged age was 24 years, with 49% male and 86% white respondents. Respondents' state driving records were used to identify offenses during the 18 months before and after (three-year total) the telephone survey. Offenses were categorized as none or at least one, and logistic regression models were used to identify the characteristics of male and female drug-drivers. Eighty-five percent of respondents drank and 51% drank and drove in the past year (n = 2815). Lifetime marijuana use was reported by 58%, past year use by 26%, and marijuana-driving by 13% (n = 726) of respondents, with an average frequency of 21 times. Use of other drugs (uppers, downers, tranquilizers, psychedelics, crack, heroin, and other drugs) was reported. Lifetime psychedelic use was highest (16%), and heroin use was lowest (3%). Similarly, 191 used psychedelics and 38 used heroin in the past year. Among respondents reporting other drug use (n = 442) in the past year, 200 (46%) reported drug-driving an average of 16 times. There was considerable overlap among drink-driving, marijuana-driving, and other drug-driving, with 116 reporting all three in the past year. Individual characteristics were explored as predictors of drug-driving, including high-risk driving (HRD) (20-item scale), hostility (7-item scale), aggression (4-item scale), risk-taking (4-item scale), and tolerance of deviance (ToD) (10-item scale). Logistic regression models showed that more drink-driving, marijuana-driving, and drug-driving significantly predicted driving offenses for both sexes. When adjusted for psychosocial covariates, drink-driving was no longer significant for men and more HRD and hostility predicted offenses, while for women drink-driving remained significant and more HRD and hostility also predicted offenses. Marijuana-driving remained significant for men and women, and more HRD, hostility, and less ToD predicted offenses for men, while for women, more HRD predicted offenses. Drug-driving remained significant for men and women, and more HRD and hostility predicted offenses for both sexes. Further analyses will be reported.

CONCLUSION: it is apparent that a number of young adults drive after using drugs, and are more likely to have offenses than those who do not. Also, several psychosocial and behavioral measures predict drug-driving. This information could be useful in prevention and intervention programs.

Fatal Traffic Accidents in Which No Alcohol is Detected: Are Drugs Related?

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One of the issues that arises higher concern in our society is death due to road accidents, because of its high incidence in Spain. Driving under the influence of drugs that affect the central nervous system is one issue of concern in road safety. For many years, attention has primarily focused on alcohol and legal limits for blood alcohol concentration during driving have been established. However during the last years drugs other than alcohol have attracted increasing attention, due to a dramatic increase of use.

The objective of this study was to get an insight into the prevalence of medicinal and illegal drugs among car drivers, in which alcohol was not detected, killed in road accidents in Southern Spain over a 2-year period (2004-2005). During this period, the Department of Seville of the Spanish National Institute of Toxicology received a total of 952 blood samples from drivers dead in road accidents; in 491 of them no alcohol was detected. These cases are the aim of the present study.

Toxicological analyses were performed in all samples received following our laboratory normal procedures. Ethanol was analysed by means of headspace GC-FID. Screening of drugs of abuse was performed by means of homogeneous enzyme immunoassay CEDIAÒ. Then SPE (Bond-Elut, certified) was performed in all the samples and the extracts were analysed by gas chromatography with NPD, high performance liquid chromatography (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). Although the majority of the 491 cases studied, tested also negative for the presence of drugs, 120 (24.44%) cases yielded positive results for illegal drugs and medicinal substances. The illegal drugs most frequently found were cannabis (THC) (n = 21), cocaine and its metabolites (n = 20). Among psychoactive drugs, benzodiazepines were the most common (n = 53). Other psychoactive drugs found were citalopram, venlafaxine, mirtazapine and zolpidem. It is interesting to point out that midazolam (n = 29) represented approximately one half of the cases positives for benzodiazepines.

The present study shows that a significant proportion of drivers dead in road accidents were under the influence of psychoactive drugs while they have not drunk alcohol. Therefore, its confirms the need of establishing the presence or not of psychoactive drugs in all specimens received from fatal traffic accidents in order to explain, if possible, their influence on the accident's causes.

Pattern of Impaired Drivers in a Southern Nigeria City

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INTRODUCTION: Sobriety check-points is a routine practice in several developed countries especially with invention of digital breathalyzers. Findings from screened candidates have assisted in establishing policies on prevention of alcohol and drugs related road

traffic accident in such countries. The use of Blood Alcohol Concentration (BAC) value as a Global legal deterrent approach to reduce road traffic accident is well documented and scientifically acceptable. This research revealed distribution of BAC amongst commercial drivers and auto-bike riders in a Southern Nigeria city and level of law enforcement knowledge about its use.

METHODOLOGY: The target population for this research covered, Police, special marshal, commercial auto-bike riders and vehicle drivers on the highways. Screening was done randomly, in proximity to a police checkpoint with one vehicle/rider selected out of every five. A portable digital breathalyzer from Craig Medical called CA2000 with mouth pieces and complemented with straws was utilized for this research. The screening was done for five hours during the day and three hours at night for a duration of five months.

RESULTS: Data analyzed revealed that trailer drivers had highest level of BAC at (36.5%) compared to that of auto bike riders (33.25%) with 0.08 and above. There is significant diurnal variation with highest BAC recorded from 9:00 p.m. upward. Research revealed that all accident related arrest based on alcohol involvement were based on police officers direct nose sniffing of the culprit and 95.5% of the arrested victims were released without appropriate legal prosecution of the offenders, thus leading to repeated recycling of the offence. The police and Federal road safety marshals were documented at 87.5% to be totally ignorant of BAC and the skills impaired by elevated BAC.

CONCLUSIONS: Several road traffic accidents are due to elevated BAC. Ignorant on BAC on the part of the law enforcement agents like the police and FRSC officials are major contributor to avoidable accident, hence urgent capacity building for such stakeholders will save the society from alcohol related accident.

Prevalence of Alcohol in Blood Samples from People Involved in Traffic Law Violations and Traffic Accidents in Turkey

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Alcohol is one of the main causes of traffic accidents worldwide. The contribution of alcohol to traffic accidents has been evaluated in a number of studies. According to the WHO reports, traffic accidents are among the main reasons for public health damage ranked in the fifth place in undeveloped countries and in the 10th place in developed countries. Moreover, traffic accidents are the major cause of mortality in Turkey. In accordance with Turkish laws, subjects were considered to be positive when alcohol blood concentration exceeded 50 mg/100mL. Our aim is to obtain an epidemiological profile of alcohol prevalence among persons involved in traffic law violations and traffic accidents in İzmir. The cases were compiled on the basis of persons involved in traffic violations and traffic accidents who admitted to the Emergency Medicine Department for an injury caused by traffic accidents. 664 blood specimens which had been collected for 18 months were screened for alcohol using by CEDIA and positive cases were confirmed by HS-GC/MS SPME method. The cases were classified into four groups; car accidents (CAs), road accidents (RAs), motor vehicle accidents (MVAs), and traffic check control (TCC). Of the 664 positive cases, 83.4% were male and 16.6% female. Of the total traffic cases, CAs cases were 28.3% (n = 188) and 17% (n = 32) of them were alcohol positive. The mean blood alcohol concentration (BAC) for positive cases was 132.3 mg/dL. RAs cases were 10.5% (n = 70) and 11.4% (n = 8) of them were alcohol positive, the mean BAC was 227.87. MVAs cases were 7.2% (n = 48) and 12.5% (n = 6) of them were alcohol positive, the mean BAC was 116.2. TCC cases were 53.9% (n = 358) and 87.9% (n = 315) of them were found to be alcohol positive. The mean BAC was 78.55. In the consideration of TCC it is worth noting that the number of male alcohol positive cases approximately 7 times higher than that of females. While their alcohol levels within groups didn't show any significant differences. It can be noted that the mean value (BAC) of alcohol positive samples in all traffic cases were found to be exceeded the legal limit concentration and a very high degree of alcohol prevalence particularly in TCC cases.

Roadside Survey of Alcohol and Drugs in Norway – Data Collection and Analysis

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BACKGROUND: The prevalence of alcohol among car drivers in Norway was surveyed in 1981-82, but the prevalence of other psycho-active substances among drivers was not known until a roadside survey was carried out in 2005-2006 by the Norwegian Institute of Public Health –FHI - in cooperation with the Institute of Transport Economics – TØI, and the Central Mobile Police Service - CMPS. The objective of this paper is to present the data collection and statistical analysis methods and problems of this roadside survey as well as some preliminary results.

METHODS: 10,000 drivers would be sufficient to achieve reasonably precise estimates of prevalence, but breaking down into subgroups would have required a much larger sample. A team from FHI collected saliva samples and asked a set of questions to a random sample of drivers on the highways in South-

Eastern Norway from April 2005 through March 2006. 360 data collection sessions were planned, with a target number of drivers varying from 15 to 60 roughly in proportion to the traffic volume at each point of time and road section. All days of the week and all hours of the day were covered. The CMPS stopped the drivers, tested them for alcohol and asked them to participate anonymously in the study. In total 26 psycho-active substances, including alcohol, medical and illicit drugs were checked. The data will be weighted in proportion to traffic volumes in each road section, time of year, week and day.

DATA OBTAINED: 349 data collection sessions or 97% were carried out as planned. The number of drivers in most sessions was close to the target, but varied from 20% to 320% of the target. In total 12,194 drivers were asked to participate in the study. 1,359 drivers, 11.1%, refused to participate, i.e. 11,835 drivers or 88.9% gave their informed consent to participate. The non-response rate was varied from 2% to 16% between police districts and periods of the year.

RESULTS: About 5% (unweighted) of the drivers providing a specimen had at least one of the drugs in their saliva. This figure showed no correlation with the non-response rate. Prevalence estimates weighted for traffic volumes will be presented in the full paper.

CONCLUSIONS: The data collection plan and procedures worked as intended, and saliva samples from almost 11,000 drivers were obtained. The non-response rate of 11% is comparable to or lower than in most previous studies. In the planning of road-side surveys all issues that can influence the number of non-responses should be carefully considered to keep this rate as low as possible. Prevalence results will provide useful information for policy makers in road safety. financed by the Norwegian Directorate for Health and Social Affairs and the Norwegian Ministry of Transport and Communications

Traffic and Alcohol: A Study on Alcohol-Related Traffic Accident Deaths in Sao Paulo

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Alcohol consumption by drivers is one of the main causes of traffic accidents, often with fatal victims. Brazilian studies which relate the involvement of alcohol and traffic deaths are rare. These data are important for the implementation of public policies capable of diminishing the number of victims and also the elevated costs of accidents. Our objective was to determine the prevalence of alcohol use by fatal victims of traffic accidents autopsied at the Medico-Legal Institute of the state of Sao Paulo.

Data on 3,042 victims killed in traffic accidents, between January and December of 2005, were collected. Sex, age, type of accident (collision or run-over) and blood alcohol concentration (BAC) were studied. In the sample studied, 43.95% of the victims had a positive BAC, with a mean value of 1.71 g/L (grams of ethanol per liter of blood). Men represented 85.9% of the cases, and 47.8% of those had consumed alcohol. Among women, 21.2% had a positive BAC at the time of death. Almost half (48.5%) of the victims were aged 25 to 44. There was a statistically significant difference between pedestrians and car occupants, in regards to age and BAC distribution.

From the study we concluded that nearly half of all fatal victims had ingested alcohol before the accidents, that the mean BAC was almost 3 times the maximum legal limit for driving in Brazil and that pedestrians who were killed were, on average, 10 years older and had drunk 1.2 times the amount that car occupants had.

Establishing BAC Profile of Bike Transportation Workers in Rural Nigerian Communities

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Nigeria is a fast growing economy. About 80% of the entire populations live in rural areas. Food crops are cultivated by rural dwellers and transported on bicycles, motorcycles and occasionally 4-wheel vehicles to rural markets, towns and cities for sale. The commonest and fastest means of transport used are the motorcycles (popularly called 'Okada' in Nigeria). The roads from the farms to the markets are narrow, tortuous, rough, sandy and at times muddy. To confront the task of riding on these rough roads, some transporters engage in the use of alcoholic beverages. At times the ooze of alcohol from the breath of the rider is palpably nauseating. Occasionally, the passenger may be given no choice of another bike than to board the drunk rider. Police and hospital records contain a large number of motorbike accidents. This study was carried out to: 1/Establish the BAC profile of bike transport workers; 2/Study the level of BAC that impairs bike riding tasks; 3/Provide an alcohol use inventory in public bike parks.

METHODS: Four motorbike parks were selected for the survey. 10 volunteers were enrolled in each bike park by simple random sampling. Each subject was evaluated for ability to drive through rough, sandy and muddy earth roads, and reaction time to unexpected events (using a cloth-banner as obstacle in a sharp bend). The digital alcohol breath analyzer (CA2000) was used to record the BAC of participants. BAC readings were documented for each participant before and at the end of the day's work. On the spot level of alcohol use was

also established for each registered member of the study population.

RESULTS: About 65% of public bike transportation workers in Nigeria operate their business under the influence of alcohol, two-thirds of this group having very significant BAC of 0.02 - 0.03.
Significant impairment of maintenance of straight course movement on a sandy terrain occurred with BAC of 0.03 in 60% of bike transport operators.
Only 35% of bike transport workers are free from detectable BAC. The locally brewed illicit gin is the commonest source of alcohol.

CONCLUSIONS: Majority of bike transport workers in rural Nigerian communities operate under the influence of alcohol.

Realities of Alcohol Consumption on Traffic Safety: A Survey of the Rate of Alcohol Use in Nigerian Motor Parks

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Among the many businesses in Nigeria, the beer industry is the next most lucrative only after crude oil. Producers of alcoholic beverages have the most sophisticated and aggressive marketing strategy known in my domain. Even in the remotest community where you may not get biscuit/bread to buy, you will get beer to buy. Government appears to be interested only in the taxes paid by the producers. Local gin (Kaikai, Ogogoro, Akpeteshi) are found every where. 96% of households have a bottle or more of the so called 'roots', (a preparation of dry illicit gin and herbal roots). Laws exist to regulate the preparation, sale, distribution and consumption of these products especially in public places but are hardly enforceable. The sale of these alcoholic beverages is booming as they help to add to the income of many households who are suffering from different levels of degrading poverty and deprivation. Again different measures of these local preps come very cheap (N10, N5) compared to the standard cost of beer (N140) per bottle. Public transport workers have access to this stuff without restriction. Expectedly, sales outlets spring up around motor parks and schools and other public places where customers are abundant. The motor park is one such place. The purpose of this study is to determine the rate of alcohol use in Nigerian motor parks.

METHOD: The questionnaire as well as the face to face method of interview was adopted in the survey. A total of 46 drivers were interviewed. The questions were few and very brief. Three busy parks were used as interview stations. 5 days each were spent at each station. Some drivers were interviewed while waiting at the motor park for their vehicles to be loaded, and others while drinking alcohol. Questionnaires were also administered on 30 other community members chosen at random.

RESULTS: 43.5% of drivers are happy for the easy access to alcohol and 56% of them use one form of alcoholic beverage or the other: 28% occasionally, 18% frequently, 10% always. 32.6% said alcohol gives them a mental state of preparedness for the driving task. 13% drink because it makes them to be bold, while 17.4% declared that they can go any length to buy alcohol. However, 80% of respondents drawn from the community want the sale of alcohol beverages banned from motor parks.

CONCLUSIONS: A significant number of Nigerian road transport workers drive under the influence of alcohol.

Drug effects on drivers

Effets des stupéfiants et des psychotropes sur les conducteurs

Residual Effects of Zopiclone 7.5 mg and Temazepam 20 mg on Actual Driving in Healthy Elderly Volunteers

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OBJECTIVE: A major problem of hypnotic drug use is residual sedation the morning after bedtime administration. This constitutes a particular safety hazard for patients who drive a car the next morning. Information on the severity of residual effects is mainly derived from studies conducted with young healthy volunteers. However, the majority of users of hypnotics are elderly, i.e. 55 years or older, who may have an increased sensitivity to the residual effects of hypnotics. Still, no studies exist assessing the residual effects of hypnotics on actual driving performance in this population.
The aim of this study was, therefore, to investigate the residual effects of the benzodiazepine hypnotic temazepam 20 mg and the non-benzodiazepine hypnotic, zopiclone 7.5 mg, on actual driving performance in healthy elderly subjects. These drugs were selected because they are among the most frequently prescribed hypnotics in many countries, and comparable studies have been conducted with zopiclone 7.5 mg in young volunteers.

METHODS: A total of 18 healthy elderly volunteers (mean age ± SD: 64.3 ± 4.4 years, range 56 - 73) participated in a double-blind, three-way crossover study. Subjects received single oral doses of zopiclone 7.5 mg, temazepam 20 mg, and placebo at bedtime. Cognitive tests of tracking, divided attention, inhibitory control and memory were conducted the following morning, starting 8:45 hrs and 11:45 hrs after administration. Between 10 to 11 hrs after administration a standardized

driving test on a primary highway in normal traffic was performed.

RESULTS: Driving performance was significantly impaired after administration of zopiclone 7.5 mg ($p < 0.001$), but not after temazepam 20 mg. Delayed recall and recognition of verbal information, and inhibitory control were significantly worse following zopiclone administration, when compared with placebo. Temazepam 20 mg did not result in a significant impairment of cognition, compared with placebo.

CONCLUSIONS: Bedtime administration of zopiclone 7.5 mg causes a significant deterioration of driving performance the next morning in healthy elderly. In contrast, temazepam 20 mg was free from residual effects.

The absence of residual effects of temazepam 20 mg and the severity of residual effects of zopiclone 7.5 mg on driving was similar to that found in previous studies with healthy young volunteers. This does not support the hypothesis that healthy elderly people, at least until the age of 75 years, are more sensitive to residual effects of hypnotics on driving than young volunteers.

The Effect of Background Music, of Varying Tempo, on General Driving Performance while Sober and Under the Influence of Alcohol

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Although the adjustment of musical devices while driving has been shown to cause distraction and beget mistakes, the playing of music is arguably harmless. It is proposed that a driver's musical preferences, habitual behaviours and immediate mood collectively determine the impact that music will have. Moreover, given that attentional resources are limited by the consumption of alcohol, the additional demand of processing musical information is assumed to result in significant impairment of driving ability. This study endeavoured to qualify the effect, if any, of in-vehicle music on simulated driving performance both while sober and under the influence of alcohol.

Young (18-21 y, n = 16) and mature (25-35 y, n = 16) participants, both male and female, were required to drive a STISIM 400 simulator, both in a sober condition and after receiving a modest dose of alcohol (0.7 g/Kg for males and 0.6 g/Kg for females). Experimental sessions comprised of 3 x 5 min simulation runs, during which either fast tempo music (Elvis vs JXL – "A Little Less Conversation", 116 bpm), slow tempo music (Nick Cave – "Let It Be", 68 bpm) or no music was played in the background at 80 dB. Participants were instructed to drive in a usual manner while abiding by speed limits, negotiating curves and responding to peripheral divided attention cues.

Mature participants attained a mean peak blood alcohol concentration (BAC) of 0.060 g/100 mL at 60 minutes after starting to drink (coinciding with the third experimental condition), while young participants reached a mean BAC of 0.055 g/100 mL. There was no statistically significant difference between the two groups in this respect.

The results of this experiment highlighted the impact listening to music while driving. The presence of music elicited a change in general driving performance for all parameters except mean speed of travel. The observed changes were manifest as relative impairment in all but one task, the divided attention task, in which an improvement was observed in the combined music condition. Music tempo was also shown to affect a number of driving parameters, observed as significant interaction effects with age or alcohol consumption.

In conclusion, in this study, music caused impairment of most aspects of driving, although the absolute effect of music tempo proved difficult to elucidate. In addition, alcohol increased lane drifting and discriminately affected speed control depending on the tempo of the music.

Relationship Between Alprazolam or Clonazepam and Clinical Impairment Among Suspected Drugged Drivers in Norway

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OBJECTIVE: Alprazolam is an increasingly prescribed benzodiazepine that relieves anxiety. Clonazepam is a benzodiazepine used to treat epilepsy. Both drugs are believed to affect the ability to drive a motor vehicle. However, few driving studies have been performed (1, 2, 3). Our Institute analyses blood from suspected drugged drivers that have been clinical examined by a physician. The aim of our study was to test if there was any relationship between the blood alprazolam or clonazepam concentration and clinical impairment judged by the CTI (clinical test of impairment).

METHODS: Blood from suspected drivers is screened for narcotics, ethanol and certain drugs at the Division of Forensic Toxicology and Drug Abuse. All results are stored together with the results of the CTI in a local database. We searched this database between December 1999 and April 2006 for blood from suspected drugged drivers containing alprazolam or clonazepam. Blood containing alprazolam or clonazepam as the only drug present was included in our study. The blood had been screened by LC-MS and immunological method and the amount of drug present were confirmed by a slightly altered LC-MS method with cut-off 0.03 µmol/L for both alprazolam and clonazepam.

RESULTS: In the actual time period 46 out of 62,712 cases (0.07%) contained alprazolam as the

only proven drug and 63 out of 62,988 cases (0.10%) contained clonazepam as the only proven drug. However, a much larger number of drivers had ingested either alprazolam or clonazepam in combination with other drugs, see table 1. Suspected drivers were judged as impaired in more than 75% of the cases at all concentration levels for both benzodiazepines, see figure 1. Independent of concentration level 91% (31 out of 34) of the cases containing alprazolam alone and 82% (40 out of 49) of the cases containing clonazepam alone were judged as impaired. There is a tendency that increasing concentrations of either alprazolam or clonazepam enhance the degree of impairment judged by the physician, see figure 2.

CONCLUSIONS: Few suspected drugged drivers had only alprazolam or clonazepam detected in their blood in the actual period. Most drivers had supratherapeutic concentrations of alprazolam in blood, while few had clonazepam levels above the reported therapeutic level.

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Adolescent Marijuana- and Alcohol-Impaired Driving Behaviours

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Marijuana- and alcohol-impaired driving behaviours were investigated among 307 Grade 9 to 12 students from predominantly rural areas of Eastern Ontario, Canada. Students were administered a questionnaire that asked about their past year marijuana and alcohol use, driving history, past year frequency of driving within an hour of using marijuana, drinking two or more drinks of alcohol, and using both substances, and how often in the previous 12 months they rode as a passenger with someone who drove under these same conditions. Similar rates of driving at least once within an hour of using marijuana (21%) and alcohol (20%) were observed among the 162 youth with a driver's license. Six percent of licensed drivers reported driving at least once within an hour of using both substances. Among licensed drivers, non- and infrequent users of marijuana and alcohol, respectively, drove less often within an hour of using each respective substance as compared to frequent users who smoke or drank more than once per month (ps < .01). Non-users of marijuana also drove less often within an hour of using both substances than did frequent users ($p < .01$). Forty-six percent of the sample reported riding at least once as a passenger with someone who drove within an hour of drinking alcohol, whereas 40% reported doing so with a driver who drove within an hour of using marijuana. Nearly a quarter (24%) of the adolescents indicated that they had ridden at least once with someone who drove within an hour of consuming both drugs. A unique aspect of this work was the investigation of differences in rates of riding as a passenger with an impaired driver according to youth's frequency of marijuana and alcohol use. Results revealed that non- and infrequent users of marijuana reported riding less often with someone who drove within an hour of using marijuana as compared to frequent users (ps < .0001). Non-users of marijuana also indicated that they rode less often with someone under the influence of both marijuana and alcohol than did frequent users ($p < .01$). Infrequent drinkers were found to ride less often with drivers who drove within an hour of drinking alcohol ($p < .01$), using marijuana ($p < .0001$), and both drugs ($p < .0001$) than were frequent drinkers. Overall, the findings from this study suggest that frequent users of marijuana and alcohol are not only more likely to drive while under the influence of these substances, but they are also more apt to ride as a passenger with someone who is under the influence. This work also extends previous inquiry on youth impaired driving by providing data on driving behaviours following the combined use of marijuana and alcohol. Implications for public policy and program development are discussed.

Driving Under the Influence of Cannabis, Reckless Driving and Accident Involvement

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Cannabis use is an increasingly common phenomenon among youth; as such, the detrimental social consequences related to cannabis consumption deserve serious considerations. The incidence of driving under the influence of cannabis (DUIC) is exceedingly high among cannabis users. Studies investigating the impact of DUIC on traffic safety concluded, that in the acute period of intoxication, cannabis reduces specific driving faculties and increases responsibility for traffic crashes. However, individuals driving under the influence of cannabis seem to exhibit a general reckless driving style contributing to an over-estimation of collisions among DUIC drivers. The study's aim was to investigate the reckless driving habits of DUIC drivers by means of self-reports and observations in a driving simulator. DUIC was further associated with the probability of being involved in a collision while controlling for potential confounding variables (age, driving exposure, reckless driving and driving under the influence of alcohol). For intervention purposes, personality traits were studied in relation to DUIC.

Participants (n = 85) were men aged between 17 and 50 years of age (mean = 27.31) recruited by Internet advertisements. They completed self-report questionnaires including personality traits (Sensation

seeking scale Zuckerman-V and NEO-PI-R), reckless driving habits (Dula Dangerous Driving Index), the number of on-road collisions over the previous three years, DUIC and driving under the influence of alcohol. Thereafter, participants completed a task consisting of a car pursuit in a dynamic driving simulator. Measures observed were collisions, mean and maximum speed.
In total, 30 participants were cannabis users 80% of whom reported at least one incidence of DUIC in the previous 12 months. DUIC was significantly associated with deliberate risky driving (r = 0.32) and negative emotion while driving (r = 0.3), but not with aggressive driving (r = 0.1). Hierarchical logistic regression analyses demonstrated that after controlling for age, exposure to driving (km/year), driving under the influence of alcohol and reckless driving, DUIC increased the probability (odds ratio: 3.24, confidence intervals: 1.02 - 10.3) of having been involved in an on-road collision. After controlling for age and driving exposure, multiple analyses of variance indicated that cannabis users (M = 8.61, SD = 7.37) have significantly ($p < 0.01$; $\eta^2 = 0.092$) more crashes in a driving pursuit task than do non-cannabis users (M = 5.79, SD = 4.48). Maximum speed in the driving simulator was higher for DUIC drivers; however, statistical analyses did not revealed a significant difference. Multiple regression analyses indicated that sensation seeking ($\beta = 0.49$) and impulsivity ($\beta = 0.26$) account for 30% of variance of DUIC.
The results suggest that DUIC is associated with a general reckless driving style. However, DUIC drivers do not seem to directly express their anger while driving. It seems that DUIC is associated with an increased probability to be involved in self-reported car accidents, even after controlling for confounding factors. These results were supported by the observations made in the driving simulator. Finally, DUIC is associated with specific personality traits (i.e. sensation seeking and impulsivity). Interventions strategies should be adapted accordingly.

Simulator Test Bed for Testing Effects of Alcohol and MDMA on Driving Performance

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The number of accidents that can be attributed to psychoactive substances is constantly at a high level. Since drug- and medicine use is proportionally increasing over the years, special efforts have to be directed towards gaining better knowledge of the various aspects of this problem and developing appropriate solutions. The objective of the recently started EU-project DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) is to give scientific support to the EU transport policy (White Paper) by establishing guidelines and measures to combat impaired driving. In the framework of DRUID, a series of experiments will be carried out in driving simulators, in various places to assess the effects of alcohol and Methylenedioxymethamphetamine (MDMA) on driving performance. In order to standardize the experiments across laboratories, a standard virtual world including relevant scenarios for testing effects of alcohol and MDMA is proposed.
Driving simulator: Participants in the experiments will be required to complete test-rides in a (fixed-base) driving simulator consisting of a mock-up car with original controls linked to a dedicated graphics computer, registering driver behaviour while computing the road environment and dynamic traffic at high rate. Participants have a 180º view of the road environment. Other vehicles in the simulated world interact with the simulator car autonomously and behave according to hierarchically structured decision rules that are based on human driving behaviour. Four types of measures will be registered during the experiment: performance, physiology, self-reports, and control measures.
Performance measures:Performance will be assessed by measuring speed or accuracy, reflecting skills at the operational and tactical levels. Speed of lead- and following during car-following are analysed on coherence, resulting in a measure of delay in response to speed changes. In addition lateral and longitudinal vehicle control, frequently used measures for vehicle handling, are analysed. Both measures have shown to be sensitive to medicinal drugs. Theories of drug use have proposed links with risk taking and impulsivity, particularly among polydrug users. To pre-empt the effects of risk taking on driver performance, gap acceptance will be assessed. Additionally, participants' response to a traffic light turning yellow will be assessed. Impulsivity will be pre-empted by assessing the reaction to critical events, for example an abruptly appearing car in a city driving scenario.
On the motorway, participants' reaction time will be measured in a situation in which traffic intensity will gradually increase until lead traffic has to brake to a standstill. This scenario arises a few times and can lead to crashes, i.e. the ultimate measure of unsafe driving.

Event-related Potentials and Secondary Task Performance During Simulated Driving

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Inattention and distraction account for a substantial number of traffic accidents. Therefore, we examined the impact of secondary task performance (an auditory oddball task) on a simulated driving task (lane keeping).

Twenty healthy participants performed two 20-minute tests in the Divided Attention Steering Simulator (DASS). A secondary (distracting) oddball task was presented in isolation and simultaneously with the driving task, to assess their mutual interference. In addition to performance measures (lane keeping in the primary driving task and reaction speed in the secondary oddball task), brain activity, i.e. event-related potentials (ERPs), was recorded. The secondary oddball task was presented in an active version (n = 10), which means that the participant had to respond to the deviant target stimuli, or passive version (n = 10), which means that no response was required.
Driving performance was not affected by the secondary auditory task, not during the active oddball task ($F(1,9) = 1.21$, n.s.), nor during the passive oddball task ($F(1,9) < 1$, n.s.). Performance parameters on the active secondary oddball task did not differ between performance of the oddball task in isolation and simultaneous performance of the oddball task with the driving task. However, when the oddball task and driving task were performed simultaneously, reaction time variability increased in the secondary oddball task ($F(1,9) = 7.58$, $p < .05$). Analysis of brain activity indicated that ERP amplitude (P3a amplitude) related to the passive secondary oddball task, was significantly reduced when the task was performed simultaneously with the driving test ($F(1,9) = 32.12$, $p < .001$).
This study shows that when performing a simple secondary task during driving, performance of the driving task and this secondary task are both unaffected. However, analysis of brain activity shows reduced cortical processing of irrelevant, potentially distracting stimuli from the secondary task during driving

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"Can a Positive THC Metabolite (THC-COOH) in Urine Be Used to Prove Impairment in a Driving Under the Influence (DUID) of Cannabis Case?"

J.R. ZETTL

A 21-year-old male was stopped for speeding at 12 a.m. midnight on Interstate 70 in Colorado, USA at Floyd Hill, which has a very steep - 6% - grade. Arresting officer observed that the subject's "speech was slow and thick tongued and that he had a brown-green coating on his tongue". Due to some dental surgery the subject stated he had taken a Vicodin earlier in the day. His right hand was in a very heavy plaster cast and indeed he had had recent oral surgery. Subject voluntarily agreed to perform Standardized Field Sobriety Tests (SFSTs), and according to the arresting officer's notes the subject "failed to perform them as a sober person would have" and arrested him for Driving Under the Influence. During a search of the subject's vehicle the officer found a partially opened 24 pack of beer with an opened can in the center console, and a glass pipe. A Drug Recognition Expert was not called to do any additional testing. A single BREATH Alcohol test was conducted using an Intoxilyzer Model 5000EN at 1:26 a.m. with a result of 0.034%. A reading of 0.034% in Colorado is under the statutory limit for DUI. A URINE sample was then collected which screened positive for Cannabinoids (Detection Limit 25 ng/mL) and confirmed for Delta-9-THC-COOH (Detection Limit 5 ng/mL) with a Cannabinoids Semi-Quant of 84 ng/mL. The urine also screened positive for Opiates (Detection Limit) 300 ng/mL) - confirmed positive for Hydrocodone.
The subject and persons he spent the day with confirmed he had consumed a MINIMAL amount of beer prior to the stop but had not consumed any other drugs than the Vicodin within the prior 6 hours. The Vicodin was provided by his dentist for pain.
Question – Should a Toxicologist be allowed to give expert testimony that the drug and drug metabolite found in the subject's urine substantiated that he was impaired to such a degree that he could not safely operate a motor vehicle?
From a Prosecution Perspective - the combination of the alcohol, failure to pass the SFSTs, speeding and drugs in his system from consumption some time prior to the event would be cause to conclude that his driving ability was affected to such a degree that he could not safely operate a motor vehicle. Urine drug concentrations can be used to infer that ones driving ability was affected.
From a Defense Perspective - Positive Urine Drug Concentrations only infer that a drug was ingested at some prior time (See DOT HS publication 809 642) and as such should never be used to attempt to show subject impairment at time of driving. Further the SFSTs were conducted on a 6% incline with cars whizzing very close by and with a heavy cast and other pain. From a purely toxicology standpoint is it generally accepted that drugs found in urine have no impairing affects on a person's Central Nervous System or ability to safely operate a motor vehicle.

Note: Drug(s) in a person's blood at or above a certain concentration can generally be used to show impairment.

Forensic alcohol analysis
Dosage de l'alcool en toxicologie médico-légale

Volpe Center Breath Alcohol Sample Simulator

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Evaluation of police evidential breath testers intended for the production of reliable evidence that can be accepted by courts (pattern evaluation) is performed for the Federal Government by the National Highway Traffic Safety Administration (NHTSA) at the U.S.

Department of Transportation Volpe Center in Cambridge Massachusetts. Evaluations are performed according to the NHTSA Model Specifications for Evidential Breath Testers. Some other countries that perform pattern evaluations use Recommendation 126 of the Organization International de Metrologie Legal (OIML). Because of the difficulty in obtaining human subject samples representative of the driving drinking population, and the associated cost, inconvenience, and sometimes unreliability of human subjects, neither NHTSA nor OIML rely on the use of actual drinking human subjects as a source for alcoholic air test samples. Mechanical devices (referred to in the OIML document as test rigs) which produce human-like test samples are used instead. At the Volpe Center, tests to determine the efficiency of capturing and measuring the human breath are done using a test rig device called the BASS (Breath Alcohol Sample Simulator). However, most of the other tests performed at the Volpe Center to determine precision and accuracy under expected conditions of use are done using the ordinary "simulator", a simple device consisting of a thermostated alcohol solution through which air is passed, and which is in wide use by police in the US. Like the human subject, and like the "simulator", the BASS device produces test samples that follow Henry's Law as the samples are generated. After the sample has passed out of the human subject, or the simulator, or the BASS and into the breath tester, the normal "ideal gas law" for non-condensable gases is applicable. Unlike test samples from the human subject, test samples from simulators and the BASS device are highly reproducible. However, unlike ordinary simulators, the BASS device produces test samples which duplicate the range of delivery rates, alcohol concentration profiles, and pressure profiles typical of drinking human subjects. The BASS device uses computer controlled metering valves which regulate the delivery of air from a compressed air cylinder through thermostated reservoirs of (1) pure water and (2) aqueous alcohol solutions which are mixed before being passed into the breath tester under evaluation.

Evaluation Evidential Breath-Alcohol Analyzer for Mobile Use in Police Cars

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Dräger Alcotest 7110 MK III-Finland was approved for testing breath alcohol to measure alcohol (ethanol) levels for legal purposes in 1998. Since then, it has been used under stable environmental conditions at police stations. Portable evidential breath-alcohol analyzer makes it possible to measure breath-alcohol level on the place of arrest at the roadside without any unnecessary delay. Transport of suspects from the roadside to the police station or hospital requires considerable police time and resources. Particularly in Northern Finland, the distance to nearest police station or hospital can be long. The aim of the study was to assure that there was an agreement of results between breath-alcohol analyzer in mobile use at a police car and in police station. Hundred and twenty-seven healthy volunteer suspect drunken drivers gave two parallel breath samples to both analyzers (police station and police car) complying with the same accepted protocol. In addition, 35 healthy volunteer police officers consumed an amount of alcohol (0.3 and 1.3 g/Kg) and breath measures were carried out within 7 hours. One to nine breath measures to both analyzers were randomly carried out in the police group. The age of volunteers (suspect drunken drivers and police officers) varied between 19 and 69 years. The study was carried out at two different cities (Helsinki and Jyväskylä), since various outdoor climate conditions were needed. Environmental conditions (humidity and temperature) were collected inside the car by Rotronic hygrolog - data collection device. During the study period the temperature inside the car varied between 6 and 34.7°C (mean value 20.4°C) and relative humidity varied between 7.4 and 70.4% (mean value 37.6%). Statistical analyses were carried out using linear modeling and statistics with R. No systematic difference was observed between alcohol values in the car and police station. Statistically significant difference, however, was found between the first and second breath alcohol measures regardless of the place of breath analyzer. But when using average elimination rate of alcohol (0.13% per hour) to correct the difference in time between the first and second measures, the observed difference disappeared.

It can be concluded that there were no differences in the results of evidential breath analyzers in mobile use at a police car and in police station under environmental conditions studied. Thus, the results of evidential breath analyzer in mobile use at police car are competent and, therefore, suitable for use in legal purposes.

Advances in Firmware Data Collection and Evaluation: Alabama's Approach

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Today legal challenges to breath alcohol testing evidence are commonplace and vary in complexity. While sometimes these challenges are supported by legal precedence or firmly established scientific principles, often times excerpts or partial offerings of reasonable

scientific theories are packaged in such a way as to seemingly negate moderately high breath alcohol concentrations (BrAC). To an alarming extent whimsical and fanciful theories have been accepted by our nation's courts as legitimate explanations for the difference in measured BrAC and the expected BrAC based on the defendant's assertion as to the amount of alcohol that was consumed. Modern breath alcohol testing instruments offer data collection and storage capabilities that were not available in instruments 10 to 20 years ago. By incorporating these expanded capabilities into its breath testing program, the objective data generated has allowed the Alabama Department of Forensic Sciences Breath Alcohol Testing Program to assess the merit of many of the common legal challenges. Increased data collection increases knowledge and allows one to verify that the instrument is performing as it is supposed to. This increased knowledge in turn yields increased confidence in the testing process. Storage and review of critical operating parameters just prior to and just after the collection of the breath samples proves that no critical error or malfunction occurred during the breath test. Storage of breath curve data from the infrared detector allows for the pre-trial review of the breath curve profile to verify the absence of mouth alcohol. Storage and review of the breath curve data from the infrared and electrochemical cell detector allows one to verify that there was no radio frequency interference. Finally, collection and storage of the flow data gives one the ability to assess subject cooperation. An overview of the unique data collection capabilities provided by the firmware utilized with Alabama's Draeger Alcotest 7110 MKIII-C will be presented.

Accuracy of Portable Breath Analyzers

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Breath alcohol analyzers have been used in Poland for more than 20 years. The police are equipped with portable, hand-held devices and stationary instruments. The first are used mostly at the roadside. In many circumstances the positive result (Breath Alcohol Concentration BrAC > 0.10 mg/L) obtained by such instrument have to be confirmed using stationary breath analyzer or blood analysis. On the other hand, three of them (Alcotest 7410, AlcoSensor IV and AlcoQuant 3020) were approved by the State Central Office of Measures according to procedures patterned on OIML (International Organization of Legal Metrology) R 126 Recommendations and their metrological properties are verified every 6 months. The aim of the study was to assess the accuracy of portable analyzers on the basis of real measurements of alcohol concentration in drunken drivers.

The results of breath and blood analyses for the study were achieved by the analysis of expert opinions concerning drunk driving elaborated at the Institute of Forensic Research in 2002-2007. In 370 cases the defendant was tested by breath analyzer and the result was confirmed by the same or/and another breath analyzer or/and blood analyses. The studies were limited to two portable instruments, Alcotest 7410 (Dräger) and AlcoSensor IV (Intoximeters, Inc). The results obtained by portable devices were compared with the readings of stationary breath analyzers (Alcomat, Siemens and Alkometr A2.0, AWAT) and blood analyses. Due to alcohol elimination process, the results of second and subsequent measurements were re-calculated using mean elimination rates. These values were estimated on the basis of differences in alcohol concentration between samples collected in time period higher than 45 minutes and amounted to 0.156 ± 0.099 g/L/h for blood (n = 206) and 0.091 ± 0.054 mg/L/h for breath (n = 149). The readings of portable instruments were in very good agreement with the results of confirmatory analyses performed by stationary devices (Alcotest 7410: $r = 0.981$, $p < 0.001$, $y = 0.993 x + 0.033$, $n = 108$; AlcoSensor IV: $r = 0.973$, $p < 0.001$, $y = 0.959 x + 0.042$, $n = 13$). The correlations were weaker when compared with the results of blood analyses (Alcotest 7410: $r = 0.932$, $p < 0.001$, $y = 1.679 x + 0.258$, $n = 259$; AlcoSensor IV: $r = 0.946$, $p < 0.001$, $y = 1.764 x + 0.156$, $n = 59$), but comparable with correlations between the readings of stationary devices and the results of blood analyses (Alkometr A2.0: $r = 0.946$, $p < 0.001$, $y = 1.793 x + 0.096$, $n = 216$; Alcomat: $r = 0.945$, $p < 0.001$, $y = 1.915 x + 0.002$, $n = 181$). The relatively high values of intercept for both portable devices could be caused by changes in blood/breath ratio of alcohol concentration with time (the portable devices are usually used first, at the roadside, when alcohol is often still absorbed into the body of the tested person). In order to compare the readings of portable and stationary breath analyzers, the Bland–Altman plots for both absolute and relative differences were also prepared. The obtained data show that the differences in results are independent of alcohol concentration both for Alcotest 7410 (absolute difference [mg/L]: $r = -0.061$, $p > 0.1$, $y = -0.0119 x - 0.021$; relative difference [%]: $r = 0.014$, $p > 0.1$, $y = 0.58 x – 4.89$) and AlcoSensor IV ($r = 0.064$, $p > 0.1$, $y = 0.0150 x - 0.024$; $r = -0.15$, $p > 0.1$, $y = -5.35 x + 2.50$ for absolute and relative difference, respectively).

The results indicate good correlation between the readings of portable breath alcohol analyzers and the results of confirmatory analyses using both stationary breath analyzers and blood analysis. It means that if a police officer follows proper procedure and the metrological properties of a breath analyzer are periodically verified, the readings of portable instruments are accurate and can be used for forensic purposes. On the other hand, the confirmatory analyses have to be performed as it is common in forensic toxicology.

Driver Performance and Accident Risk of Patients with ADHD

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The paper presents the results of study within the European Immortal project on Assessment of fitness to drive amongst patients with learning difficulties. Undoubtedly, mental status and cognitive skills are important requirements for driving. Yet scientific research has not been able to point out a causal relationship between such parameters and safety of driving for drivers with Attention Deficit Hyperactivity Disorder (ADHD). The main objective of the study presented here has been to explore how specific dysfunction of ADHD is associated to driver performance and accident risk ADHD Study. The principal characteristics of ADHD are inattention, hyperactivity, and impulsivity. The skills needed for safe driving are several; the ability to focus on the road, attention to detail, and sustained attention are often the areas which all patients with ADHD have difficulty. Becoming distracted by the passengers in the car, by the radio, or something outside the vehicle for even just a moment, can cause an accident.

The present study is the first Norwegian study on ADHD and car driving. On an international level there are few studies on ADHD and fitness to drive. In the present ADHD study, seventeen adults with ADHD participated and completed the tests without side effects. Stimulant medication was used in a double blinded design to ensure evaluation under both conditions. A high end-simulator was used as an instrument to investigate the driving skills of patients with ADHD in comparison to a control group and in terms of influence of adherent medication (Ritalin) for the diagnosis. Subjective workload was measured in the study, using the NASA RTLX.

When data from the driving simulator were analysed, the most striking finding is the similarities between the ADHD group and the reference group. There are small differences with regard to distance to vehicle in front or shift in position in the lane. These differences indicate that persons in the ADHD group show better driving skills. However, the differences are small and not considered to be of practical significance.

The data in this study compared a reference group of drivers with patients in medicated or non-medicated condition during a navigation task in a high-end simulator. The results indicate that there are not distinct and clear group differences, either between reference drivers and patients with active medication or compared to patients with placebo treatment. Separating the ADHD drivers into two groups, denoting them as fast or slow drivers, some interesting results on subgroups and effects of medication surfaced. A correlation analysis suggest that the cluster label follows the medical condition, that is, subjects tend to be e.g. fast drivers when medicated, then become labelled slow when the placebo treatment condition. A possible interpretation of this pattern can be that the subject compensates their medical condition in accordance to their driving. This may be the case for fast drivers when driving without medication: They slow down in the placebo treatment in order to get everything right. Concerning the slow drivers, there may be another mechanism at work. This change of driving performance (from slow to fast) may possibly be a more traditional ADHD pattern, in which placebo treatment suffers the impulse control and thus results in faster driving. The simulator performance showed no clear differences between the ADHD group and the reference group. At the same time we know that persons with ADHD have more car accidents and make more traffic violations. We also see that the selected ADHD group in this study has more accident involvement than the reference group. The most probable reason for our findings is the test situation in the simulator. When there are guiding clues and fairly interesting tasks, often persons with ADHD can perform very well, even on vigilance tests. Our data indicate that the participants with ADHD were quite cautious and very attentive during the tests.

This ADHD study shows that individual factors may play a role in fitness to drive and that there may be individual response to medication.

Residual Effects of Flunitrazepam, Zopiclone and Zolpidem in Elderly Drivers Submitted to Simulated Driving Accidents

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In Europe, not only the proportion but also the number of older drivers increases regularly. Even in absence of pathology, this population commonly experiences changes in the motor, sensory and cognitive abilities necessary to drive. These changes are usually small but highly interactive and additive which can result in marked modifications in efficiency. Moreover, about 13% of old people are sedative or hypnotic users, due to the fact that sleep disorders and insomnia increase with age. Epidemiological data reveal that, in both young and elderly users of benzodiazepine hypnotics with a long half-life, traffic accident risks significantly increase.

These molecules can in fact lead to drowsiness related to their residual effects. To remedy these undesirable side-effects, other families of hypnotic drugs, such as imidazopyridines or cyclopyrrolones, have been developed. Experimental studies have shown that these latter present fewer residual effects due to their short half-life and their fixation sites, which differ from those of traditional benzodiazepines but, strangely, the majority of experimental studies have been conducted in healthy young volunteers.
We therefore performed an experiment with a view to testing any residual effects of these hypnotics on elderly drivers' capacities. We compared the effects of zopiclone (7.5 mg) and zolpidem (10 mg) with those of flunitrazepam (1 mg), used as a reference benzodiazepine, against a placebo. This was double-blind cross-over study. The subjects (experienced drivers between 55 and 65 years) underwent four experimental sessions separated by a washout period of at least 15 days. Subjects received one capsule at 11:00 p.m. on the day before each session, under the supervision of an experimenter. The next morning, the subject was brought to the experimentation room, 10 hours after taking the drug, and was submitted to a driving test. The task was to drive, as usual, in an urban environment where accidents scenarios were introduced. Accidents scenarios were inspired from prototypical situations which were implemented by data from real accident cases. Subjects thus had to react to sudden events and also to events involving more attention.
Preliminary analysis seems to show modifications of driving behaviour the day after taking an active molecule. Results will be discussed as such, and relative to those obtained with monotonous driving tasks generally used to test the effects of such drugs.

Hypnotics Drugs Residual Effects on Monotonous Simulated Driving in Elderly Drivers

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Old people, and particularly women, represent a large part of drivers. It was demonstrated that, due to pharmacokinetic modifications, the risk of driving accidents is increased in this part of population. To date, no study on healthy old people was made to compare residual effects of zolpidem and zopiclone, largely prescribed, in particular in France. The purpose of this work is to characterise the residual effects of zolpidem and zopiclone on monotonous car driving assessed using a driving simulator in elderly subjects by comparing these effects with those of a reference hypnotic drug (flunitrazepam) and a placebo. Monotonous car driving is widely used to assess residual effects of drugs, and showed previously residual effects of zopiclone in particular, in healthy young subjects.
This study is carried out on 16 healthy elderly volunteers (age range 55-65 years, experienced drivers). At 11:00 p.m. the day before each session, the subject took a tablet of either zolpidem 10 mg, zopiclone 7.5 mg, flunitrazepam 1mg or a placebo. The study was conducted according to a balanced, double blind, cross-over design. Each subject followed four sessions held at intervals of at least two weeks. The test drive involves driving for 1 hour along a two-lane road in a rural environment. No other vehicle or pedestrian is represented. Driving is performed in daylight. The instructions were as follows: 1) to ensure maximum lateral stability of the vehicle, 2) to respect driving at 110 km/h. At no time during the session did the subjects receive stimulation by actions external to the driving operation.
Results will be compared to those obtained in healthy young subjects to quantify risk of driving a car in elderly subjects after taking hypnotic drugs.

Traffic Accident Risks Associated with the Prescription of Antidepressants: A Registry-based Cohort Study

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Several experimental studies have shown that both depression itself and the use of antidepressants can impair ability to drive a motor vehicle. Population-based studies have been inconclusive.
The aim of the present study was to examine whether the use of antidepressants by drivers increases the risk of being involved in traffic accidents. Between January 2004 and September 2006, information on prescriptions, road accidents and emigrations/deaths was obtained from three Norwegian population-based registries. Data on people between the ages 18 - 70 (3.1 million) were linked for the three databases using the unique 11-digit identification number assigned to all individuals residing in Norway. Exposure consisted of receiving prescriptions for antidepressant use. Standardized incidence ratios (SIR) were calculated by comparing the incidence of accidents during time exposed (e.g. for the first seven days after filling a prescription) with the incidence over the time not so exposed.

Sedating antidepressants (tricyclic antidepressants, mianserin and mirtazepine) were studied together as one group and newer non-sedating antidepressants (SSRIs, moclobemide, venlafaxine and reboxetine) as another. The SIR was calculated for the overall study population, as well as stratified by age groups and genders. 22,405 road accidents with personal injuries occurred during the study period including 133 with exposure to antidepressants. The traffic accident risk did not increase for drivers who had received prescriptions for sedating antidepressants (SIR 1.1; 95% CI: 0.7 - 1.6), but only for drivers receiving non-sedating antidepressants (SIR 1.8; 1.5 - 2.2). The SIR estimates for male drivers were higher than for female drivers, and higher for middle aged (35 - 54 years of age) than for older drivers. SIR estimates did not change substantially for different time periods after dispensing of the prescription, for concomitant use of other impairing drugs, or for new users.

There was an increased risk of being involved in a traffic accident after having received a prescription for non-sedating newer antidepressants, but not for older, sedating antidepressants. The increase was modest, not higher than what has been seen for receiving any drug (SIR 1.7; 1.6 - 1.8). Further analysis will be presented to investigate whether the increased accident risk was due to the antidepressants themselves, to co-medication or to the disease it self (confounding by indication). Time of day for accident and probable drug intake will also be discussed.

Using Responsibility Analysis to Evaluate Fatal Accident Risk for Drivers in Québec Who Used Drugs

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In studying how alcohol and drugs contribute to road accident risk, researchers are often confronted with a lack of exposure data for accidents. Case-control analysis is appropriate for evaluating accident risks based on exposure to various drugs. However, obtaining a biological sample that is comparable between the case group and the control group is a major problem. Other problems are precisely identifying the drugs used by an individual and evaluating the effects of various drugs on the ability to drive a vehicle. Methods to analyze responsibility have been developed to circumvent these problems, but accident risk does not seem to be estimated correctly using these methods. The present study, carried out using a sample of driver deaths from 1999 through 2002, concludes that the estimated odds ratio corresponds to the risk of being responsible for an accident rather than to the accident risk itself. Responsibility was analyzed by both a panel of judges and by using an evaluation grid. There were some differences between the two methods used, but the estimated risks are almost the same for each method, although the odds ratios are generally slightly higher for the panel of judges, without being statistically significant. Both analysis methods often come to the same conclusion as to the level of responsibility, that is, in 80% of the cases, but the grid method indicates a larger proportion of cases that only partially contributed. The panel of judges' method generally shows the same cases to be responsible. However, the proportion of cases judged to be responsible is very high regardless of the method used, which causes difficulties in estimating risk. Risk estimation is also difficult because of the large number of sub-groups of drugs. Still, this methodology is a very interesting approach for analyzing road safety problems. Thus, in the present study, we note that the risk of being responsible for an accident increases for drivers in which alcohol, cannabis, benzodiazepines (tranquillizers and sleeping pills) and cocaine were detected. However, when cannabis and benzodiazepines were detected alone, the estimated odds ratios were not significantly different from nil. The drug most frequently detected is alcohol, but cannabis, benzodiazepines and cocaine were also found in the deceased drivers. Using more than one drug seems to be very frequent in the population studied. Finally, and somewhat surprisingly, results from urine samples or blood samples are comparable.

A Short Series of Toluene Impaired Drivers

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The abuse of volatile compounds is commonly referred to as "huffing". Often the subject saturates a rag with solvent, places it over the mouth, or inhales or sprays the volatile substance directly into the mouth resulting in altered consciousness. Tolueneis among the most frequently abuse inhalants, found in solvents, paints and other products. Onset of symptoms occurs within seconds to minutes following inhalation and produces an intense euphoria followed by sedation and unconsciousness.

We report here a series of six toluene impaired drivers who were evaluated by Drug Recognition Evaluation (DRE) officers. Blood toluene concentrations were determined by headspace GC, with headspace GC-MS confirmation. The relative retention time (RRT) of toluene to the n-propanol internal standard of 3.77 and 2.84 on 2 different systems, (ethanol RRT is 0.61 and 0.57, respectively). In each case toluene was the only impairing substance identified. All six subjects were males and their ages ranged from 25 – 55 (mean 36 years) and had the blood toluene concentrations ranged from 12 – 45 mg/L (mean 24 mg/L). The half-life of toluene in blood is 13 - 68 hours. A 1979

study of toluene abusers described significant signs of intoxication in subjects with blood concentrations of 1 - 2.5 mg/L. Half of those with blood concentrations between 2.5 – 10 mg/L were hospitalized for marked intoxication. Two of the subjects we encountered were contacted after motor vehicle crashes. Three were stopped for severe erratic driving, and one for failing to stop at a red light. In all cases, impairment was very obvious; subjects had slurred speech, red, bloodshot watery eyes, appeared severely intoxicated. Solvent abuse was suspected due to an obvious chemical odor. One subject had gold paint all over his face. All but one subject were candid as to their methods and frequency of abusing the inhalant. For those who performed the DRE evaluation, there were inconsistencies on performance. Subjects generally did poorly on the walk and turn test. One subject was unable to keep his head still long enough to complete the HGN test, however the remaining five subjects had six of six clues present. Four subjects attempted the convergence test and all exhibited a lack of convergence. The results on the remaining tests were not consistent, for example 4 of 6 subjects completed the Romberg Balance test and of these, 2 exhibited fast internal clock, while 2 were very slow. Similarly, there were inconsistent observations on heart rate, blood pressure, pupil size and muscle tone. All subjects admitted to huffing in the car, and made statements which indicated that it was their practice to do so while driving, because the effects wore off rapidly. This group is older than the stereotypical young adult inhalant abuser. The blood concentrations of these cases were much higher than earlier reports. This is consistent with longer term inhalant abuse and several of theses subjects did indicate they had been huffing for years. From the treatment literature inhalant dependent adults have the poorest prognosis for recovery.

The Effect of Sleep Deprivation, and Acute d-amphetamine and d-methamphetamine Administration on Visual Field Function: An Event-Related Potential Study

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INTRODUCTION: While the epidemiology literature highlights an association between road crashes and both amphetamine use and sleepiness, the mechanisms responsible for this association are yet to be determined. This uncertainly is particularly important in relation to amphetamines, where experimental cognitive research indicates that amphetamines generally have cognitive enhancing properties. A possible mechanism may relate to the effect that both amphetamine use and sleepiness have on peripheral visual field functioning. The present research examined the acute effects of low-level d-amphetamine and d-methamphetamine, as well as the effects of 27 hours sleep deprivation (SD), on aspects of visual field processing using a sensitive measure of brain functioning, event-related potentials (ERPs).

METHODS: Two, double-blind, placebo-controlled, counterbalanced cross-over studies were performed where twenty healthy participants attended two testing sessions separated by one week, and were administered i) placebo, and ii) 0.42 mg/Kg amphetamine (d-amphetamine in the first and d-methamphetamine in the second study). A third, counterbalanced, crossover design was performed where twenty healthy participants attended two testing sessions, separated by one week, where they either had a normal night sleep or 27 hours SD. In each session of each study, participants completed a simple visual discrimination task (in which the location of target and non-target stimuli was manipulated to activate central and peripheral visual field processing separately), while behavioural and ERP data were recorded.

RESULTS: Across the visual field, accuracy and reaction time measures were not affected by low-level d-amphetamine or d-methamphetamine, but they were impaired by 27 hours SD. ERP measures of early sensory (posterior P1/N1) and attentional processing (vertex N100, frontal N200) were not affected by either amphetamine or SD. ERP measures of later cognitive processing (P300) were not affected by amphetamine, but were reduced with SD. The above results were not related to visual field position.

CONCLUSIONS: This study suggests that low-level amphetamine does not affect simple sensory or cognitive processing, regardless of visual field position. Further research is required to determine whether these results are generalisable to the moderate to high levels typically encountered in impaired drivers. Conversely, SD impaired higher cognitive function and resultant performance measures, with no effect on early sensory or cognitive processing detected. These results were not related to visual field position, which suggests that the SD impairment seen in sleep deprived drivers is not due to a specific peripheral impairment, but may represent a more general impairment of higher cognitive processing.

Observations on Pupil Sizes of Drug Users and its Applicability to the Drug Evaluation and Classification Criterion

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In this study, optometric values from proprietary data were used to examine one of twelve factors that is examined during the DEC (Drug Evaluation and Classification), the pupil size of the suspect. While

pupillary size has been established as a parameter that can be affected by drug usage, currently the training officers are taught that pupils are "normal" irrespective of the lighting condition. Further, the DRE (Drug Recognition Experts) are taught that pupils are "generally normal" with presence of certain drug classes such as PCP. This subjective blanketed description taught to officers is deceptive, as there are other factors such as age and iris color that may affect pupil size possibly skewing the now predicted pupil size. The purpose of this study to examine the following: (1) determine whether objective optometric values obtained from DRE can be a predictor of a specific drug classification, (2) determine the affects of poly-drug combinations on optometric values, and (3) examine age and iris color on the prediction of drug classification.

Six hundred and fifty six cases were examined from various police departments; however, much of the data could not be used due to discrepancies with the paperwork. Pupillary measurements and toxicology results from casework that could be used in the study were put into a statistical database, SPSS. ANOVA and chi-square analysis were performed on the single drug cases to reflect whether arrestees positive for a single drug pupil sizes were different from a negative population that was generated from the data. Further, to reflect if poly-drug users had a distinctive pupil size compared to the negative population ANOVA analysis was performed on combination drug arrestees.

The limited data used did corroborate with the predicted pupil size for THC, CNS depressant, narcotic analgesic, and PCP positive cases. However the data did not support the DEC predicted pupil size in CNS stimulant users, but this can be due to the limited amount of data and among other factors, such as toxicology issues, limiting this study. The data also did not allow a study on age or iris color due to the heavily biased data to ages under thirty and brown iris population. Certain drug combinations such as THC with PCP (called "sherms"), CNS stimulant with narcotic analgesic (often called "speed balling"), CNS stimulant with depressant, and THC with CNS stimulant were also examined in this study. The statistical analysis indicated that drug combinations yield pupil sizes that are not distinct from single drug cases, thus polydrug combinations could be problematic for the DRE to predict.

It should be mentioned that DREs make their decisions based on the totality of the tests given as part of the DEC protocol. Pupil sizes or any one aspect of the DEC alone doesn't enable the officer to discern impairment or drug classification. However, by examining this study it can be inferred whether other sections of the DEC can be corroborated with objective values collected from the field.

Alcohol, drugs and traffic safety
Alcool, médicaments, stupéfiants et sécurité routière

Case Law vs. Scientific Testimony

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The New Mexico Department of Health Scientific Laboratory Division's Toxicology Bureau is tasked with overseeing the breath and blood alcohol testing for drugs and alcohol for the State's law enforcement agencies and for the Office of the Medical Investigator. On an annual basis, the laboratory analyzes about 2,500 blood cases from law enforcement and about 2,500 cases from the Office of the Medical Investigator for alcohol content. We currently oversee over 300 breath alcohol testing instruments which had over 45,000 breath tests conducted on them. As the regulatory agency for the breath and blood alcohol testing that is done through-out the state, laboratory staff is tasked to testify in the adjudication of DWI/DUI cases.

There are various case laws that have been passed in New Mexico that tackle the issues of DWI and how they are approached in a court of law. There have been case laws for the admission of evidence (i.e.; State v. Alberico, 1993); how breath testing is handled (i.e.: State v. Onsurez, 2002 State v. Christmas, 2001; State v. Gardner, 1998); and how blood alcohol tests are handled and maintaining chain of custody [i.e.: State v. Christian, 1995; State v. Dedman, 2004; State v. Silago (issues of retrograde extrapolation), 2005]. The majority of these cases have facilitated Toxicology staff in testifying in court. In June of 2006, case law from the New Mexico Court of Appeals issued a ruling (State v. Day, 2006) that made testifying for these cases much harder. The Court of Appeals ruled that for cases that had results that were around our per se (0.08) and aggravated (0.16) DWI levels, retrograde extrapolation and/or impairment would be needed due to the fact that DWI in New Mexico is charged for time of driving. This put tremendous burden on the four Forensic Toxicologists that had to testify as expert witnesses through out the state because the blood alcohol analysis report form (SLD 705 form) is not only a chain of custody form but it also is accepted in the State's magistrate and municipal courts. Thus, the court case load increased because now the toxicologists had to testify in cases in lower courts, increasing court testimony by 75%. In the spring of 2007 the New Mexico Legislative Session considered this issue and passed legislation that would ease the burden on the toxicologists. This legislation modified the crime of driving under the influence of intoxicating liquor to allow three hours for the administration of a

chemical test to determine alcohol concentration. It also provides for the admissibility of chemical tests taken more than three hours after driving.
The goal of this presentation is to bring to the fore the issues that the State Toxicology lab has had to overcome judicially. It is also the hope that by bringing these issues to the fore, other law enforcement, judicial, and scientific agencies will be able to use this information to better handle DWI cases in their areas.

Head Injuries in Road Traffic Accidents

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Head injury is recognized as a major public health problem and those due to road traffic accidents account for the great majority world wide.

AIM OF THE WORK: This study had been designed to evaluate the epidemiology of head injuries in road traffic accidents among trauma patients attending Assiut University Hospitals. This is a prospective hospital based study and was conducted in the Casualty Department of Assiut University Hospitals during the period from April 2004 to April 2005.

RESULTS: The total number of head injured cases were 1,331 out of 43,310 (total number of trauma patients) with an incidence of 3.07%. Head injuries due to road traffic accidents represented 60.9% (810 cases). 35.8% of cases (290) were in age group of 20 -< 30 years, followed by the age group of 10 -< 20 (22.2%) and 30 -< 40 (18.52%), the least affected age group was that of age > 60 (4.9%) and < 10 (2.5%). Males represented 85.7% and females 14.3%. Most of victims in road traffic accidents were pedestrians (52.6%) followed by drivers (31.6%) and passengers (15.8%). In 1,100 patients out of 1,331, head injury was associated with other severe trauma or major bone fracture in other body regions i.e. 231 were pure head injuries. 182 of patients with pure head injuries were due to road traffic accidents, 43 of them had lacerated wounds in the scalp and the radiological examination revealed nothing. The remaining patients (139), the radiological findings varied from skull fracture [36.7%: linear (29.5%), fracture base (2.2%) and depressed fracture (5%)], brain contusion (28.7%), hematoma [23%: epidural (7.2%), subdural (11.5%) and intracerebral (4.3%) and diffuse brain injury [33.1%: diffuse axonal injury (7.2%), subarachnoid hemorrhage (14.4%) and diffuse swelling (11.5%)]. More than one radiological finding may be present. Patients with radiological findings (139) were classified according to Glasgow Coma Scale (GCS) into: severe (GCS < 8) 32%, moderate (GCS 9-12) 22% mild (GCS 13-15) 46%. They were also classified according to Glasgow Outcome Scale (GOS) into: recovered patients (57.6%), patients with moderate disability (9.4%), and those with severe disability (3.6%), vegetative state (7.2%) and death occurred in 22.3% of patients. The relation between GCS and Glasgow Outcome Scale (GOS) revealed that: Complete recovery occurred in 93.7% of cases with GCS 13-15 while recovery was not recorded among patients with GCS < 8. Moderate disability was detected in 23.3% with GCS 9-12, severe disability was 6.7% in GCS < 8 and the same percentage in GCS 9-12, vegetative state 22.2% in GCS < 8 and didn't recorded in the other GCS. Death occurred in 66.6% of patients with GCS < 8 and 3.3% in GCS 9-12. Causes of death were evaluated and found to be circulatory failure (16.13%), brain death (32.26%), multiple organ failure (35.58%), and other causes (16.13%).

CONCLUSIONS: Road traffic accidents have become the first public hazard in the world, which results in one of the largest threats against human lives and safety. In this prospective hospital based study the incidence of head injuries was found to be 3.07%. 60.9% of head injuries were due to road traffic accidents. The highest incidence was in the age group of 20-<30 years (35.8%). They were more common among males and among pedestrians. Pure head injuries were 231 victims. 182 patients of pure head injuries were due to road traffic accidents, 43 of them had only lacerated wounds in the scalp, while 139 patients revealed radiological findings. The victims of pure head injuries due to road traffic accidents were classified according to GCS and GOS and the relation between both scales was evaluated.

Deficits in OSAS and Neurological Patients - Influence in Traffic Safety

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INTRODUCTION: Apart from a multitude of physical complaints, obstructive sleep apnea syndrome (OSAS) patients and neurological patients suffer from excessive daytime drowsiness, reduced sustained attention, limited memory processes and cognitive functions. Important factors in this can be the severity of the disorder on the one hand, and the duration of prior therapy on the other hand. Among other aspects, such a decline in performance influences the persons affected in their ability to drive a car.

ISSUE: The objective of this study was to investigate the influence of these symptoms and the effect of nCPAP therapy on OSAS patients resp. neurological rehabilitation on patients suffering from stroke, cerbrovascular diseases, brain trauma, brain tumor

etc. During admission to the clinic, these patients were examined neurologically and neuropsychologically and were tested e.g. with Carda, a driving simulator (vigilance), which was developed and standardized at Clinic Ambrock. Measurement of vigilance was invariably carried out in the afternoon and took place in a quiet, sound-proofed room.

FINDINGS: Testing of vigilance achievements revealed a highly significant difference between healthy persons and OSAS patients ($p < .001$) resp. neurological patients ($p < .001$). After more than 6 weeks nCPAP resp. 3 weeks of neurological rehabilitation, the OSAS patients resp. the neurological patients' quality of life improve to a significant degree (both patient groups: $p < .001$). Analysis of the degree of severity showed for OSAS patients no significant difference, for neurological patients a significant difference between mild and severe ($p = .020$) (concerning vigilance achievements). The comparison between different clinical kinds of neurological disorders concerning vigilance and the research of influence of the OSAS and of the neurological diseases on driving fitness (traffic safety) is yet to be carried out.

DISCUSSION: The study revealed that patients with OSAS and neurological diseases show problems and deficits concerning their vigilance achievements and their memory processes. The improvement of vigilance achievements and memory processes should show a lower driving fitness (traffic safety) in untreated patients and increasing traffic safety in patients under rehabilitation.

Evaluation of DUI Offenders: An Examination of the Quebec Compulsory Protocol

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Research has shown that a high percentage of DUI first-time offenders will repeat. However it remains difficult to pinpoint the characteristics of first-time offenders and to predict the risk of recidivism. In Québec, since 2002, new legal and administrative measures have lead to the development of an evaluation protocol. This protocol aims at detecting the risk of recidivism among primary offenders. This report is part of an on going evaluation program that also includes four other sections. The analyses are aimed at providing Québec with the best available detection protocol with regard to the prevention of DUI recidivism.

This evaluation protocol was developed and implemented by the umbrella association of the addiction treatment centers in Québec – the FQCRPAT. Following a structured interview and the administration of validated detection tests, the results are analyzed against a decisional flowchart. On the basis of the results, the examiner makes a recommendation to the agency responsible for the delivery of drivers' license, i.e., the Société d'assurance automobile du Québec. The recommendation is to the effect of pursuing or not pursuing the assessment. If the examiner emits an unfavourable recommendation, the driver is then submitted to a complete assessment to determine the level of his needs. A remedial plan is then set and needs to be followed thought to regain one's drivers' license. The driver is responsible for all the costs incurred by an unfavourable recommendation.

With the current protocol, 15% of the drivers have received an unfavourable recommendation. Data collected pertain to the drivers' characteristics (sex, age, level of education, civil status, housing, employment), driving habits (other infractions, blood-alcohol level at the time of the arrest), drinking habits (frequency, quantity, type of products used, people and places related to consumption, results of the validated tests used in the assessment protocol), the circumstances leading to their arrest. In the presentation, we will discuss the strengths and limitations of detection measures and the flowchart and also discuss the process involved in the evaluation protocol.

Prevention and intervention
Prévention et intervention

Decision Making in Medical Psychological Assessment Procedures: Translation of the Assessment Criteria in Reports on Fitness-to-Drive

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OBJECTIVES: In Germany the medical psychological assessment of the driver's aptitude (MPA) is a mandatory condition for license reinstatement in any case the DUI offender scores a BAC equal or above 1.6 mg/g or has repeated offenses in the past. Since the Federal Highway Research Institute has published the Expert Guidelines on Driver's Aptitude in 2000 the medical and psychological preconditions for re-licensing apply accordingly for these assessments. In 2005 the Assessment Criteria (Schubert & Mattern, 2005) were released and introduced as a standard document in order to support the process of decision making. The development of these criteria additionally tended to make the individual assessment process comprehensible and fair. This study aimed (1) to analyze to what extent the criteria and the postulated indicators were translated in the reports on fitness-to-drive, (2) to identify the

client's statements and on-site findings that were taken into account as diagnostic information and (3) how the relevant information were integrated to a final result and diagnostic judgement.

METHODS: 35 reports on fitness-to-drive from 4 different assessment institutes were randomly chosen and analyzed by two especially educated evaluators (Kappa: .75 - .95).

RESULTS: The results indicate that most of the decisions were indeed comprehensible, but some indicators (e.g. for problem awareness, cooperativeness, need for abstinence, quality and stability of behavioral changes) were not concretely operationalized and thus are poorly checkable (e.g. "and"-connection in one indicator, vague descriptions of alcohol amounts or missing time details). Only a few of the indicators that should be explored actually were explored. In most cases it was traceable by means of which statements the diagnostic information was gained, but the manner of interpretation for decision making remained subjective to a large extent. In addition to that it became clear that numerous indicators are not empirically approved or do not comply with the current state of the art.

DISCUSSION: The results of the evaluation show that a reliable and statistically testable algorithm for the integration of diagnostic information in order to make the decision verifiable is missing (Schuhfried, 2004). The findings indicate a substantial need for further research on this topic.

After Two Years of per se Legislation in Switzerland: Prevalence of Drugs Among Drivers in Geneva

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Switzerland has introduced in 2005 a so called "per se" traffic safety law with a zero tolerance for major illicit drugs (THC, cocaine, morphine, and amphetamines). This law states that "a driver is considered unable to drive each time it is proved that his blood contains" one of the above-mentioned substances. The Swiss Society of Legal Medicine has proposed cut-offs at 1.5 ng/mL for THC and 15 ng/mL for the other substances in whole blood, in order to have equity all over Switzerland. Although the previous law included enough rules to punish intoxicated drivers, this new regulation encouraged police forces to ask for more toxicological analyses. At the same time, the blood alcohol concentration limit has been reduced from 0.8 to 0.5 g/Kg. However, only cases above 0.8 g/Kg are considered as a major traffic offense. In DUI cases urine is analyzed by immunological and chromatographic methods in order to assess the substances consumed by the driver. Identified substances are then quantitated in blood in order to determine if the driver was under the influence at the time he/she was driving. The analyses focus on drugs-of-abuse.The total number of DUI cases analyzed by our Institute increased from 450 in 2003-2004 to 997 in 2005-2006. DUI cases included only drivers suspected to be under the influence of other drugs than alcohol. In those cases a urine sample is taken in addition to blood sample. The average age of drivers was stable at 34 ± 14 and blood alcohol concentration (BAC) remained exactly the same at 1.28 ± 0.61 g/Kg. During the first period (2003-2004), 55% of the drivers (62% during the second period) had BAC above 0.8 g/kg whereas 9% (5%) were between 0.5 and 0.8 g/kg. 69% (57%) of the drivers were positive for drug(s) other than alcohol. Cannabis was the most detected substance in urine with 53% (38%) of the cases being positive. Cocaine was present in 22% (17%), benzodiazepines in 14% (13%), opiates in 8% (12%), methadone in 7% (6%) and amphetamines in 2% (4%). Other detected substances included barbiturates, methaqualone, dextropropoxyphene, tricyclic antidepressants, and other psychoactive drugs. Among the drivers positive for cannabis in urine, 69% where found positive also in blood during the first period (75% during the second period). For cocaine, 64% were positive (57%), for amphetamines 75% (50%), and for morphine 31% (49%). When drugs-of-abuse where positive, other drugs were not determined in blood. The total number of BAC determinations increased by 23% whereas the percentage of positive cases (82%) remained stable. Cases with low BAC (between 0.5 and 0.8 g/Kg) also remained stable, because the breathalyzer result is evidential if the driver accepts the value.

The comparison of the data obtained along those two periods, before and after the new law came into force, shows that the number of cases increased as the police could more easily prosecute intoxicated drivers and because their attention was focused on this issue. The higher number of cases did not provide more positive results. The reduction of the BAC limit did not provide more cases because the driver who accepts the breathalyzer result is not submitted to any blood sampling.

Heroin Use in Drug Cases Reviewed for Driver Licensing

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Driver licensing in Great Britain is dealt with at the centralised Driver and Vehicle Licensing Agency (DVLA). The Drivers Medical Group processes around 500,000 cases a year from individuals in whom there is at least preliminary evidence that they may have a medical condition that could affect their fitness to drive. Included, are those which require assessment for drug misuse or drug dependency.

The Regional Laboratory for Toxicology, City Hospital,

Birmingham UK, undertook the analysis of urine specimens to determine drug misuse in 8,076 cases for the DVLA between July 2002 and December 2006. Out of the 8,076 cases analysed, 24 tested positive for heroin, as determined by the presence of the primary heroin metabolite, 6 monoacetylmorphine (6 MAM). Of these 24 cases, 23 were male. Ages ranged from 20 - 52 and the majority (17 cases) were in the range 26 - 40. Multiple drug use involving cannabis, cocaine, and methadone was identified in 6 of these cases. A total of 10 of the 24 cases reviewed were identified through the High Risk Offender (HRO) Scheme. HROs are drink/drive offenders who by the nature of their drink/drive conviction(s) are required in GB regulations to undergo a medical examination on re-licensing. Of all these cases only 2 meet the prescribed medical standards of fitness and are currently able to hold a driving licence. Notifications received from the Police resulted in 4 case assessments. The others were from a variety of sources including the medical profession, taxi-licensing authorities and information obtained during routine medical enquiries.Of the 22 non-licence holders, 6 demonstrated a short-lived period of abstinence from drug use, sufficient to permit temporary re-licensing. However, at the time of renewal of this temporary licence, these drivers tested positive for 6-MAM and the licences were revoked.There were 4 first licence applications but 2 of these had already become subject to the HRO assessment, having been convicted of drink/driving offences prior to application for a driving licence. Of the 22 cases in which a licence had been refused, 11 have incurred driving endorsements whilst not holding a valid driving entitlement. These endorsements have occurred following a medical refusal/revocation of the licence, while banned by the Courts or without ever having held a valid driving licence.

From Science and Statistics to Safer Roads

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OBJECTIVE: To build on the knowledge provided through research, science and statistics and translate that knowledge into safer roads. To create an environment within which impaired driving will not be tolerated. To change driver behavior and affect a reduction in impaired driving and the subsequent injury and death caused.

METHODS: Build a base of awareness of the consequences for impaired driving; these consequences would include harm to others, and real costs to individual drivers for the offence of impaired driving (licence suspension, costs, impoundment, ignition interlock). Increase the likelihood of apprehension and the perception of same – for all drivers and road users; encourage police services and government to allocate more resources to the summer months. Use the science of the positive (social norm marketing) to motivate drivers to do the right thing: call home, take a cab, stay overnight, or designate a driver. Incorporate research that shows "everyone knows you're not supposed to drink and drive" (Atkins and DeJong) and create messaging that addresses how to plan ahead and drive sober. Work with government, police, public health, business, community groups and victims to saturate communities with this information about the right way to get home.

RESULTS: In Ontario, the arrive alive DRIVE SOBER® campaign has been built on these fundamental components for almost 20 years by Ontario Community Council on Impaired Driving (OCCID). Our marketing committee oversees focus testing and reviews messaging and liaises with others when necessary to get the job done to the very best of our capacity. Targeting the summer months, OCCID has achieved remarkable reductions in the high numbers of fatalities that used to occur all through the summer months. One example is the month of July where we once (1988) saw 61 alcohol-involved fatalities whereas in 2003 the same statistic was down to 19; August for the same time period dropped from 51 to 15. These are great reductions especially when you adjust for the 40% increase in the number of drivers licensed in Ontario during the 16-year time span. With a changed social climate towards impaired driving, OCCID has been able to secure support from high profile contacts including NHL players and musicians to enhance our public awareness messages and incorporate the findings as laid out in the DeJong Atkins study. The campaign continues to grow and is in a position to expand to address evolving areas of concern link to off-road vehicle fatalities.

CONCLUSIONS: Working with a limited budget for materials and resources ($100,000) OCCID has built the arrive alive DRIVE SOBER campaign to an amazing level and driver behavior has changed especially during the summer months. The campaign leverages just shy of $2 million annually in donated airtime and boasts the involvement of almost 400 groups, police services, schools and businesses province-wide. In Ontario, driver behavior has changed and the attitudes towards impaired driving have changed. "Friends don't let friends drink and drive" in Ontario and even other road users and passers-by will take a stand to prevent impaired driving by intervening or reporting an impaired driver.

Youth and Impaired Driving in Canada: Progress to Date

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Despite the progress that has been made, traffic crashes remain the leading cause of death among 16-25 year old Canadians. Even conservatively estimated, over 45% of these deaths are alcohol related. In 2005, MADD

Canada commissioned R. Solomon and E. Chamberlain to survey the countermeasures that the provinces and territories could implement to reduce impairment-related traffic crashes among Canadian youth. In August 2006, published "Youth and Impaired Driving in Canada: Opportunities for Progress".
The purpose of this paper is to summarize and update the 2006 study, and report on the progress that has been made in implementing its five priority recommendations: More rigorous enforcement of the existing liquor licence prohibitions against selling or providing alcohol to minors or intoxicated youth; Implementation of a comprehensive graduated licensing program (GLP) comprised of three licensing stages; Enactment of a zero BAC limit for all drivers under the age of 21 or for the first five years of driving; Enactment of broader police powers to enforce the GLP and zero BAC limits; Introduction of systematic sobriety checkpoint programs in areas frequented by young impaired drivers and pedestrians.

Staging an Intervention on Impaired Drivers

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OBJECTIVE: This paper discusses the relevance of public involvement in impaired driving prevention through detection and intervention. Operation Lookout® originated in Canada and is a community-based program with the goal of safer roads achieved through public awareness and action. When road users observe a motor vehicle being operated in an unsafe or erratic manner, they are encouraged to call 9-1-1 to report the incident as a crime in progress. The program combines key components of a successful prevention and enforcement strategy: it intervenes to remove unsafe drivers from the road before they crash and it demonstrates to all drivers that people are watching.
Operation Lookout is an effective strategy for removing all unsafe drivers from the road regardless of the source of impairment and as such can be an integral part of addressing drug-impaired and/or fatigued drivers. The program began in the early '90's and it relies mainly on support from community groups either via local committees with the sole purpose of implementing and maintaining Operation Lookout, or via an existing group that takes on the program as one of its many activities. Operation Lookout committees have been known to provide support to several other sober driving initiatives in their respective communities.

METHODS: Communities running the program use signs on their roadways, posters in stores, and other messaging including Public Service Announcements (PSAs). Groups currently running the Operation Lookout program have provided regular reports to the host organization. Our analysis of the program reflects both the information provided by communities and by relevant stakeholders and is incorporated into an Operation Manual. The program currently operates in 40 communities across Canada and has expanded to include watercraft, off-road vehicles and snowmobiles; and is offered in French.

RESULTS: Historically, significant deterrents have been in the form of police-operated spot-checks and regular police patrols; we note that communities running Operation Lookout have shown a four-fold increase in the number of impaired drivers reported to police. Operation Lookout complements enforcement programs and goes further by facilitating a mechanism for immediately reporting any unsafe driver to the police so that immediate action can be taken. Studies have shown that there has been a fundamental shift in the social climate regarding impaired driving over the last two decades making today's environment one in which offenders from the 1980s might now instead intervene to stop an impaired driver. The program has been positively reviewed in Health Canada's road safety literature as a way to address the persistent hard-core drinking driver.

CONCLUSIONS: The program is most effective when local citizens, community groups, businesses police and emergency measures personnel form a partnership to develop and implement this program within their community. In many jurisdictions, legislation has been increased to become much more rigid, suggesting that for the people who continue to drive impaired, the significant penalties and consequences are not having the intended deterrent effect. A different strategy is required to address the hard-core persistent impaired driver and Operation Lookout provides the tools for the public to intervene.

The Combined 'Zero Tolerance Law' and 'Impairment Law' for Drugs and Driving in Finland

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In February 2003, zero tolerance law for illicit drugs and driving was introduced into the drunken driving legislation in Finland. The amended law is applied to the scheduled drugs (Narcotic act 1289/93; Penal code 50). The scheduled drugs in Finland include the drugs that are listed in the UN conventions on narcotics and psychotropic substances. The zero tolerance is applied if the controlled drugs or their (active) metabolites are found in blood. The zero tolerance law is not applied if the driver has a right to use the controlled substance, e.g. a benzodiazepine by prescription of a physician.The impairment law stays still in the background in the legislation. A driver will be convicted for driving while intoxicated if the driving

ability is impaired by the use of drugs. This applies to any substance, and a driver can be convicted for the intake of any drug (of medicinal drugs, too) if he/she is intoxicated to the extent that he/she may be dangerous to traffic safety (Penal code 23). The impairment has to be proven in court. Symptoms of drug use have to be shown by documentation of a policeman and/or by a clinical sobriety test also known as clinical performance test by a physician. The impairment has to be proven also when the driver is prosecuted for 'severe drunken driving' because of drugs. The driver can be convicted for severe drunken driving because of drugs if he/she is intoxicated to the extent that he/she may be severely dangerous to traffic safety. The statutory limit for drinking and driving in Finland is 0.50% (w/w). The limit for severe drunken driving is 1.2%. The corresponding breath alcohol control limits are 0.22 mg/L and 0.53 mg/L (Law on amending no. 23 of the penal code 655/1994). Following the introduction of the 'zero tolerance law', the total number of samples in suspected drugs and driving cases increased 118% from 2002 to 2006. Increase in the positive findings of amphetamine was 255% at the national level during the same time. In the cases of illicit drugs and driving, the impairment in driving ability has not to be proven in the court anymore. Confirmation of the presence of illicit drug(s) in blood is enough for prosecuting for drugs and driving, because of drunken driving. After introducing the zero tolerance law the authorities have better means to prosecute an intoxicated driver. The police are satisfied with the zero tolerance law. Early intervention is meant to be beneficial for both the traffic safety as well as for the health and the future of the drug user.

Women Who Come to Treatment as a Result of a DUI Offense

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OBJECTIVE: This is a study of 8,464 adult women and 21,155 adult males who entered Texas substance abuse treatment between 2000 and 2005. They were on probation for driving under the influence (DUI), were referred to treatment by DUI probation, or had been arrested for DUI in the past year. They were compared on demographic characteristics, substance use patterns, DSM-IV diagnoses, and levels of impairment.

METHODS: This is a secondary analysis of an administrative dataset collected on clients in publicly-funded programs in Texas. T tests and chi square tests were used to determine significance and bivariate and multivariate models were constructed. Only findings significant at .001 are presented in this abstract.

RESULTS: The proportion of females who were sent to treatment as a result of DUI has increased from 27% in 2000 to 32% in 2005. They were more likely than males to be white (73% vs. 56%), to have used substances a shorter period of time (17 v. 19 years), to be unemployed (75% v. 56%), and to meet the DSM criteria for drug dependence (32% v. 23%). The women were more likely to be divorced with minor children (22% v. 5%) and to have come to treatment to regain custody of their children (11% vs. 2%). Males were more likely to be alcohol dependent (49% v. 44%). Women were more likely to have injected drugs (31% v. 23%), to use substances daily (42% v. 40%), and at admission to be treated for depression or anxiety (21% v. 10%). They were less likely to complete treatment (67% v 72%). They reported significantly more days of problems on the 6 domains of the ASI at both admission and at 60-day follow-up. At follow-up, they were more likely to be living with someone who abused alcohol or used drugs (9% v.7%). At discharge, 67% of the women and 72% of the men completed treatment. For women, having been in residential treatment and receiving medication for their mental health problems were the best predictors of completing treatment. At 90-day follow-up, 41% of women and 47% of men were contacted. Some 34% of the women and 39% of the men reported being abstinent in the month prior to follow-up. Those who had completed treatment were twice as likely to be abstinent and living in a household where they were exposed to alcohol abuse or drug use was the strongest predictor of not being abstinent.

CONCLUSIONS: Although females comprise only 29% of the DUI treatment admissions, they are more impaired and face more problems than their male counterparts. Additional resources, including treatment for co-occurring disorders, less chaotic living conditions after treatment, and more carefully targeted countermeasures, including closer supervision after treatment and use of vehicle control mechanisms, may be necessary to help these women achieve and maintain abstinence to prevent additional DUI episodes.

Adult Substance Use and Driving Survey-Revised (ASUDS-R): Psychometric Properties and Construct Validity

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A brief description of the Adult Substance Use and Driving Survey-Revised (ASUDS-R) is provided including the various scales and a number of items. History and development are discussed. Construct validity is set forth and psychometric properties are reviewed including content validity, scale independence, perspective, criterion and predictive validity. The use of the instrument solely or in combination with placement criteria in providing DWI education and treatment services is discussed.

The procedures involved the description of samples,

determining means, standard deviations (SD) and internal consistency reliabilities (ICR) (Cronbachs Alpha). Intercorrelations of the scales were performed along with ICR, squared multiple correlations (SMR) and percent unique variance (PUV). Correlations with age, gender, ethnicity and marital status among others demonstrate how a scale measures what it measures. Correlates between ASUDS-R scales and independent criterion measures were also reviewed in order to provide evidence of criterion (construct) validity of the scales. Included, but not limited to, were level of supervision of DWI offenders, women offenders, general drug involvement, degree of drug disruption and symptoms. Also included were levels of supervision of DWI offenders. Several other scale correlations were used including prior diagnosis of substance abuse/ dependence and driving related measures. Finally, regression analysis was performed to help determine whether the ASUDS-R is useful for placing DWI offenders in education and/or treatment.

The analytical results were largely favorable. Internal consistency reliabilities were within optimal range. Each scale was found to render a unique dimension, intercorrelations among scales showed a consistent positive manifold, consistency of measurement among different samples was strongly supported and robust correlations were found with external criterion tests and scales. Evidence was found to support the use of the ASUDS-R scales independently and in combination with collateral variables to provide service guidelines for DWI offenders.

The ASUDS-R is a valid self-report differential screening instrument that provides sound guidelines for decision making, particularly when integrating findings from other report data, e.g., BAC, prior offenses, and when used in combination with placement criteria such as those developed by the American Society of Addiction Medicine (ASAM).

Prevention of Recidivism Among First-Offenders Drunk Drivers: Three-Year Outcomes of a Randomized Controlled Trial

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About one fifth of first offender drivers arrested for driving while intoxicated (DWI) will be rearrested during the three years following the offence. Different types of interventions have proven a certain level of efficacy.

OBJECTIVES: Measuring the efficacy of different types of educative interventions that could reduce the prevalence of recidivism among first offenders arrested for DWI.

PATIENTS AND METHODS: All eligible drivers received a voluntary invitation for participating to an educational program in order to reduce the probability of recidivism. Drivers who accepted were firstly interviewed and gave their informed consent to participate. Then, they were randomized into three different groups, corresponding into three kinds of interventions: a two-hour session, a half-day session with someone they previously have chosen for accompanying them during the session and a one-day session considered as the gold standard in the French-speaking part of Switzerland. Each participant paid 250$ for participating. Each participating driver got a significant reduction of the driving licence suspension duration. Three years after the offence and the participation to the program, every driving administrative personal chart of the drivers were checked of any arrest for recidivism for DWI.

RESULTS: 1,588 drivers were eligible, 733 (46.2%) accepted to participate and 726 were formally included and randomized. 648 effectively participated to one of the three interventions (89.3% of included participants). 90.0% were males; mean age was 37.1 ± 0.9 years. The mean blood alcohol concentration when arrested was 1.58 ± 0.03. About one fifth of the sample was alcohol dependent and one third alcohol abusers. After 3 years, 85 drivers were rearrested for DWI (11.70%). Recidivism was distributed according to the type of intervention as following: two-hour session (9.2%); half-day session with a close relation (9.4%), one-day session (15.9%). Eligible drivers who refused to participate had a recidivism rate of 13.3%. Chi-square calculations confirm a significant difference between groups ($p = 0.0299$). Half of recidivism happens in the first 650 days.

CONCLUSIONS: A very brief intervention has a real impact on the recidivism rate of DWI first offenders. Less can be unexpectedly better.

Post-mortem toxicology and case reports
Toxicologie post-mortem: études de cas

Postmortem Redistribution of Basic Drugs: Comparison of Heart Blood and Femoral Vein Concentrations

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AIMS: Blood is typically the specimen of choice for detecting, quantifying and interpreting drug concentration in postmortem forensic toxicology. However, postmortem blood drug concentrations may not reflect concentrations at the time of death and may vary according to the sampling site and the interval between death and specimen collection. The volume of distribution, lipophilicity, molecular size, pH and pka of drugs are known as important factors in postmortem redistribution. In general, femoral blood is accepted as the most reliable postmortem specimen for drug analysis in forensic toxicology because the drug concentration in peripheral blood samples is closer to the antemortem concentration than that in cardiac blood. But femoral blood cannot be obtained in some cases. In the absence of the femoral blood, the most frequently used specimen is heart blood. However, the relationship between cardiac blood and femoral vein is not clearly known. The fundamental purpose of this study is to investigate comparison of drug concentrations between cardiac and peripheral blood collected from the femoral vein of the same subject.

METHODS: Blood samples (1 mL) fortified with internal standard (50 µL) and adjusted to pH 6 by the addition of 3 mL of phosphate buffer were extracted with solid phase extraction prior to analysis by GC-MS. The volatile components were removed in a stream of nitrogen. The residues were reconstituted in 100 µL of ethanol. The injection volume for GC-MS analysis was 1 µL. GC-MS analysis was performed using a Agilent MSD 5973 mass spectrometer operated in the electron-impact mode equipped with an injector operating in the splitless mode (with a 0.75-min splitless period) and a DB-5MS capillary column (30 m x 0.25 mm x 0.25 µm) using helium as carrier gas.

RESULTS: Drug concentrations were measured in postmortem femoral and heart blood samples from 12 cases. Sixteen different drugs were detected from the 12 cases. The result showed that heart/femoral blood concentration ratios were greater than 1.0 in all cases. These ratios ranged from 1.0 - 1.5 for lidocaine, propofol, doxylamine, diazepam, propranolol, benztropine, chlorpromazine and paroxetine, 1.5 - 2.0 for bupivacaine, tramadol and nordiazepam, and 2.0 or higher for amitriptyline, diazepam, fluoxetine, metoprolol, lorazepam and olanzapine.

CONCLUSIONS: This finding implies that there is no close relationship between drug properties and postmortem redistribution.

Optimization of the Determination of Carbon Monoxide in Postmortem Blood by using an IL 682 CO-Oximeter

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AIMS: Carbon monoxide (CO) poisoning is a significant cause of fire-related deaths in South Africa. The rapid increase in population in recent years has resulted in many people having to live in informal houses constructed of wood, corrugated iron or cardboard. The families utilize paraffin lamps and candles, which often result in fires, many of which are fatal. As urbanization has increased, the Forensic Chemistry Laboratory in Pretoria, South Africa has received a steady increase in the number of samples submitted for postmortem analysis of CO. Until 2006 the laboratory utilized a GC-TCD method for analysis of CO, with a separate spectrophotometric method for determination of total hemoglobin concentration. This method was found to be labour intensive and with an increasing number of blood samples, an alternative method needed to be investigated. CO-oximetry, using an IL 682 CO-oximeter with some sample pre-treatment, was the method of choice. The method is low cost, highly automated and produces a valid result within minutes.

METHODS: The IL 682 CO-oximeter has a thallium/neon hollow cathode lamp, and monitors wavelengths of 553.0 nm, 585.2 nm, 594.5 nm, 626.6 nm, 638.3 nm and 667.8 nm. Samples received for carbon monoxide determination, as well as MULTI-4™ CO-oximeter controls were used in this experiment. Sample pre-treatment involves the addition of sodium dithionite for to reduce methemoglobin to hemoglobin before aspiration into the CO-oximeter. Initially, the procedure was to add a spatula of sodium dithionite to a small amount of postmortem blood; however, this method was replaced by the more quantitative procedure of dilution with a saturated solution of sodium dithionite.

RESULTS: The procedure of just adding a spatula of dry sodium dithionite to a small amount of blood resulted in various instrument errors including “Check Cuvette”, “High MetHb” and “High Turbidity” messages. Using a saturated solution of sodium dithionite to dilute postmortem blood at a 1:1 ratio and vortex-mixing to ensure complete reduction of methemoglobin, satisfactory results were obtained. The performance

of this method on international proficiency testing specimens has been excellent.

CONCLUSIONS: This study demonstrated that, with the correct pre-treatment of postmortem blood, the CO-oximeter could be used for routine carbon monoxide determination in postmortem blood successfully, replacing the time-consuming, demanding and complicated GC-TCD and spectrophotometric method previously used.

Pitfalls in Cyanide Detection in Postmortem Toxicology: Two Case Examples

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AIMS: Forensic toxicologists should elucidate the cause of death in unknown poisoning cases, and so it is important to measure the concentrations of poisons in evidence specimen properly. However, poisons sometimes show postmortem alteration such as artifactual production, and also happened to be detected by mistake. In this presentation, we present two postmortem examples of artifactual production and mistaken detection of cyanide, and draw attention to possible pitfalls in cyanide analysis.

METHODS: Cyanide was measured by head-space gas chromatography (column: GS-Q; detection: NPD). Head-space reactions were adopted to be 30 min incubation at 50°C, under acidic conditions of 1 M phosphate buffer (pH 6.0) for coffee samples or 10% phosphoric acid and 30 mM ascorbic acid for biofluid samples, to suppress the alteration of cyanide levels.

RESULTS: In the first case, cyanide (3.2 µg/mL) was detected in an adulterated coffee drink and extensive forensic investigation revealed that isobutyl nitrite had been added. The resulting hydrolyzed nitrite had reacted with the polyphenolic ingredients in coffee, and cyanide had been produced. The second poisoning case involves a false positive detection of cyanide. Cyanide was detected in leftovers of carried rice and stomach contents using the Konig reaction without microdiffusion pretreatment, and thus positive cyanide detection could be attributed to interference of thiocyanate in the biofluids which the assay does not distinguish from cyanide.

CONCLUSIONS: Even in emergency situations in poisoning cases, forensic toxicologists should consider the validity of the analytical methods adopted and the behaviors of poisons in bodies and evidence specimens, so as not to fall into a pit of false cyanide detection.

Analysis of Mitragyna Speciosa (Kratom) Alkaloids in Human Urine as a Marker of Chronic Kratom Abuse: Preliminary Results

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AIMS: The kratom plant (Mitragyna speciosa Korth., Rubiaceae) is a tree growing up to 50 feet in height usually found in central and southern Thailand as well the northern Malaysian peninsula. Kratom has been proven to provide analgesic effects and is widely used in Thai folk medicine in the treatment of opium addiction. In Thailand the kratom plant and its alkaloids are controlled substances. The plant is readily available on the Internet. Different routes of administration include chewing green kratom leaves, smoking kratom resin or drinking tea prepared from dried kratom leaves. The aim of this study was to compare the types of alkaloids found in the urine of kratom users with the alkaloids extracted from kratom leaves and tea and to establish a marker of chronic kratom consumption.

METHODS: Four different sample preparations were analyzed: fresh leaves and tea prepared from fresh leaves (approx. 8 g), and dried leaves and tea prepared from dried leave (approx. 4 g). The samples were extracted at pH 8.5 using 25% ammonia 2 x 10 mL ethyl acetate to yield approximately 1.1%, 0.2%, 0.16% and 0.05% of crude alkaloids, respectively. Urine samples were collected from a 60 year-old self-reported chronic kratom user of 40 years with an average consumption of 50 leaves per day. Six consecutive urine samples were collected over 24 h, before and after taking 12, 25 and 30 leaves of kratom. Two extraction procedures were investigated. One 10 mL sample was alkalinized to pH 8.5 with 25% ammonia and extracted with 2 x 10 mL ethyl acetate. A second 10 mL sample was extracted at pH 9 using 1 mg sodium tetraborate and 2 x 10 mL ethyl acetate. All organic layers were dried under nitrogen and reconstituted in ethyl acetate before analysis by GC-EI/MS operating in full scan. Separations were carried out on a DB-5MS capillary column. No standards were available but the spectra could be searched against a library for identification and confirmation.

RESULTS: The alkaloids found in urine samples were similar to those found in the leaf and tea extracts. Four alkaloids found in every extraction were a 9-methoxy oxyndole alkaloid (RT 12.179, M 414), mitragynine (RT 14.777, M 398), paynantheidine (RT 14.979, M 396), and speciogynine (RT 15.297, M 398). 7-hydroxy-mitragynine (RT 12.462, M 414) was only found in the fresh leaf extract. The two major alkaloids found in the urine samples that can be used as markers of kratom consumption are mitragynine and speciogynine. The urine extraction procedures at pH 9 using sodium tetraborate and ethyl acetate gave better separation efficiency.

CONCLUSIONS: The markers for urine analysis to indicate kratom consumption are proposed. The described urine extraction procedure is simple, and provides clean extracts suitable for routine GC-MS analysis. Quantification has not yet been investigated.

A Review of Deaths Involving Methamphetamine in Utah

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AIMS: We reviewed all deaths in Utah in which methamphetamine (MA) was identified in the decedent's blood to: (1) determine whether the incidence of these deaths has changed over time; (2) examine the correlation, if any, between blood concentration of the drug and classification of death; and (3) describe demographic and toxicologic profiles of the decedents.

METHODS: Analysis for MA was conducted using GC/MS with a limit of quantitation of 0.05 mg/L. MA was detected in 525 deaths between 1995 and 2005.

RESULTS: For 88 decedents, the cases were classified as "MA-caused"; the drug's toxic effects directly caused death. For 206 deaths, the cases were classified as "MA-related"; the drug's effects contributed to the cause of death. The drug was present but did not contribute to or cause death in the remaining 231 deaths. The range of blood concentrations of MA were similar for both MA-caused (range: 0.16-10 mg/L, median: 0.39) and MA-related deaths (range: 0.07-11 mg/L, median: 0.32), with 87% of the deaths having concentrations less than 2.50 mg/L. The mode of death for 84% of MA-caused and MA-related deaths was classified as undetermined. In contrast, for cases in which MA was present but did not play a role in death, the most common modes of death were suicide (38%) and homicide (25%). From 1995 to 2000, the average annual increase in deaths involving MA was 14%, rising to 28% between 2001 and 2005. In 1995, 25% of deaths involving MA also involved other drugs. By 2005, multiple drug deaths involving MA had risen to 63%; MA was frequently combined with cocaine, heroin, and alcohol. Beginning in 2001, prescription drugs (mainly methadone and oxycodone) were also increasingly present in deaths involving MA. Eighty-five percent of deaths involving MA occurred along the Wasatch Front, the metropolitan region of Utah in which 70% of the population resides. In contrast, an analysis of numbers of deaths with respect to county population shows elevated rates in some rural counties as well. Although the average age of decedents in most deaths involving MA has remained between 21 and 54, the range of age of decedents has widened over time. The first fetal demise due to maternal MA abuse was reported in 1998 and there were 3 cases involving 63-year old decedents in 2004 and 2005. Male decedents continue to dominate, however, the proportion of female decedents increased from 9% in 1995 to 22% in 2005.

CONCLUSIONS: We conclude that both the incidence and complexity of these deaths has increased over time. In addition to blood concentrations of the drug, accurate classification of deaths involving MA requires consideration of other factors, including the possibility of polydrug use, an understanding of the changing demographic characteristics of decedents, and greater emphasis on other aspects of the autopsy investigation.

Benzodiazepines in Forensic Cases Received in 2005 and 2006

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AIMS: Benzodiazepines are common in the samples received by our department and a lot of different combination of benzodiazepines is observed in the samples. Therefore it is relevant to evaluate combinations and frequency of these substances and relate it to different subgroups. This presentation is a retrospective investigation of benzodiazepines in all samples (from autopsy cases and living persons) received by our department during two years from 2005 to 2006. The purpose is to evaluate frequency, combination and concentrations of benzodiazepines in blood and muscle and relate it to the different subgroups.

METHODS: The cases will be divided into subgroups i.e. drug addicts, traffic cases, relation to violence and criminal assault.

RESULTS: During the two years 936 samples were positive for one or more benzodiazepines. This covers about 40% of all cases received in the period. 20% of the positive cases were found in drug addict deaths, 30% were traffic cases, 10% were from violent crimes and 0.5% were rape cases .35% of the 936 samples were positive for diazepam and diazepam is then the most frequently observed benzodiazepine. After diazepam: clonazepam (27%), nitrazepam (19%), chlordiazepoxide (14%), oxazepam (14%), bromazepam (13%), flunitrazepam (12%), alprazolam (10%), zopiclone (9%) were seen. To a lesser extent lorazepam, clobazam, midazolam, phenazepam and lormetazepam were detected (< 0.5%). Diazepam (45%), bromazepam (25%) and nitrazepam (23%) were the most frequently detected in drug addicts. Benzodiazepines observed in traffic cases were often clonazepam (39%), diazepam (34%) and nitrazepam (24%). Diazepam (51%), clonazepam (36%) and bromazepam (18%) were seen frequently among violent crime. Diazepam (50%) was most frequently in combination with sexual assault.

CONCLUSIONS: Diazepam, clonazepam and nitrazepam were the most common benzodiazepines. Clonazepam was more frequently observed in traffic cases and among violent crime than in drug addicts.

A Series of 1,1-Difluoroethane Related Deaths

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AIMS: Volatile abuse is widespread among young adults and includes such agents as solvents, adhesives, petroleum products, refrigerants and propellants. Inhalants are abused for their sudden onset of intoxicating effects, euphoria, disorientation and hallucinations. These effects are frequently accompanied by nausea, vomiting, ataxia, convulsions, coma or death. Causes of death related to inhalant abuse include asphyxia, suffocation, accidents due to high risk behavior, and cardiac arrhythmias. 1,1-Difluoroethane (DFE), a halogenated hydrocarbon, has found recent popularity as an abused inhalant since it replaced the ozone-damaging chlorofluorocarbons as a propellant in compressed dusting products and keyboard cleaners. We report here a series of 9 deaths, in which use of DFE was determined to be a contributing factor in the death (Table 1).

METHODS: Postmortem blood was analyzed for volatiles by headspace gas chromatography and headspace GCMS. Drug screening by EMIT was performed for cocaine, opiates, methadone, benzodiazepines, PCP, amphetamines, barbiturates, tricyclic antidepressants, cannabinoids and propoxyphene, and by both basic and acid neutral extractions with GCMS analysis. DFE is detected in the volatile screen with a relative retention time of 0.7 when compared to ethanol. DFE was confirmed by headspace GCMS.

RESULTS AND CONCLUSIONS: DFE was not quantitated in these cases due to difficulties in preparing reliable standards and controls with this volatile gas. This limitation emphasizes the importance of complete scene investigation and autopsy to rule out other potential causes of death.

Table 1: Circumstances of Death Associated with DFE

Case	Sex	Age	Circumstances	Cause of Death	Manner of Death	Other Toxicological Findings (units mg/L unless otherwise indicated)
1	M	20	Found submerged in bathtub	Asphyxia due to drowning	Accident	Nordiazepam <0.01 , Lidocaine, Caffeine
2	F	16	Found submerged in hot tub	Asphyxia due to drowning	Accident	Caffeine
3	M	33	Found on floor of bedroom	Acute intoxication of DFE	Accident	Ethanol 0.04 gm/100 mL Carbamazepine 9.43, THC 1 ng/mL, THC-COOH 6 ng/mL, Caffeine, Nicotine
4	M	29	Found on bed	Acute intoxication of DFE & ethanol	Accident	Ethanol 0.19 gm/100 mL
5	M	17	Causing Driver in a traffic fatality	Blunt force trauma	Accident (Traffic)	THC 6 ng/mL, THC -COOH 44 ng/mL, Benzoylecgonine 0.79, EME 0.04, Chlorpheniramine 0.35 Dextromethorphan 0.17
6	M	36	Found in bedroom	Acute intoxication of DFE	Suicide	None
7	M	26	Found in a port-o-potty	Acute intoxication of DFE	Accident	Naproxen 6.75
8	M	44	Found prone on bed	Asphyxia	Undeter.	Methamphetamine 0.65, Amphetamine, Ethyl chloride
9	M	20	Found on bath-room floor	Acute intoxication of DFE	Accident	Atropine, Nicotine, Caffeine

Suicidal Drug Ingestion Involving Zaleplon

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CASE REPORT: Zaleplon (Sonata®) is a nonben–zodiazepine hypnotic from the pyrazolopyrimidine class, chemically related to zolpidem and used in the short term treatment of insomnia by decreasing the time to sleep onset. Zaleplon is available in 5 and 10 mg capsules. The recommended dosage for most adults is 10 mg, but 5 mg may be sufficient in some individuals. Zaleplon is rapidly absorbed with a time to peak concentration (t_{max}) of 1 hour and a half-life ($t_{½}$) of 1 hour. The bioavailability of zaleplon is approximately 30% and it has no active metabolites. Zaleplon exhibits CNS-depressant effects such as drowsiness, dizziness, short-term memory impairment, and difficulty with muscular coordination. We report a case of a 56 year old male nurse with a history of depression and suicidal ideation. He was found deceased in a hotel room with multiple prescription medications.

METHODS: Blood was analyzed for volatiles by headspace gas chromatography. Drug screening by EMIT was performed for cocaine, opiates, methadone, benzodiazepines, PCP, amphetamines, barbiturates, tricyclic antidepressants, cannabinoids and propoxyphene, and by both basic and acid-neutral extractions with GC and GC-MS analysis. A late eluting peak was identified as zaleplon by library match with a molecular weight of 305 amu and three major fragments at 248 m/z, 263 m/z and 219 m/z. Zaleplon quantitation was performed by liquid-liquid extraction, followed by positive electrospray ionization LC-MS in full scan with a calibration curve using 0.1, 0.25, 0.5 and 1.0 mg/L standards.

RESULTS: Zaleplon was detected in peripheral blood at 0.79 mg/L. Tissue distribution studies for zaleplon were conducted with the following findings: central blood 0.87 mg/L; bile 2.55 mg/L; liver .66 mg/Kg; vitreous 0.74 mg/L; and brain 3.35 mg/Kg. A single oral dose of zaleplon of 10 or 20 mg in healthy men produced mean peak serum levels of 0.03 and 0.04 mg/L, respectively, at 1.1 hours with an average elimination half-life of 1.0 hour. In addition to zaleplon, peripheral blood toxicology revealed ethanol 0.18 gm/100mL; buspirone 1.18 mg/L; zolpidem 0.14 mg/L; and fluoxetine 0.22 mg/L. Norfluoxetine and caffeine were reported but not quantitated.

There is one report in the literature of a multiple drug intoxication case involving zaleplon. The concentrations in this case are lower, however they are significantly higher that therapeutic. There was significant ethanol to contribute to acute CNS depression. The peripheral blood concentrations for buspirone were 10-20 times therapeutic levels, however there are no reports of fatalities due to buspirone overdose in the literature.

CONCLUSIONS: The cause of death was ruled as a multi-drug intoxication, and the manner of death, suicide.

Case Report of a Multidrug Intoxication Fatality Involving GHB

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CASE REPORT: Xyrem® (gamma-hydroxybutyrate or GHB) is a therapeutic agent used for the treatment of daytime sleepiness or cataplexy associated with narcolepsy. It is also a recreational "Rave" drug. In addition, GHB is an endogenous compound that can be artifactually elevated during the postmortem interval, which complicates interpretation.

We report the death of a 53 year old woman undergoing treatment with Xyrem® for narcolepsy. Her husband reported that she went to bed at approximately midnight, noted that she was snoring loudly, and found her unresponsive six hours later. Emergency medical personnel responded but no resuscitation efforts were made as she was clearly deceased. The decedent was prescribed tramadol, 650 mg three times per day, gabapentin, 300 mg six times per day, cetirizine 10 mg once per day, modafinil 200 mg twice per day, carisoprodol 350 mg 1-2 at bedtime and Xyrem® (GHB) 500 mg per dose. All medications had been prescribed within 1 month of the date of death and pill counts were consistent with medication compliance.

METHODS: Peripheral blood and urine were submitted to the laboratory and blood was analyzed for volatiles by headspace gas chromatography, drug screening by EMIT for cocaine, opiates, methadone, benzodiazepines, PCP, amphetamines, barbiturates, tricyclic antidepressants, cannabinoids and propoxyphene, and both basic and acid neutral extractions screens with GC and GCMS analysis. GHB was analyzed by liquid-liquid extraction procedure (without conversion to its lactone, gamma-butyrolactone (GBL)) followed by TMS derivatization and GC-EIMS. The method uses diethyleneglycol (DEG) as the internal standard.

RESULTS: Toxicological analysis of the blood revealed GHB 165.6 mg/L, tramadol 0.46 mg/L, carisoprodol 1.9 mg/L, meprobamate 3.9 mg/L, and acetaminophen 6 mg/L. Urine GHB levels were 90.7 mg/L. An intravenous dose of 3.5 g in a 70 Kg adult produced a peak blood GHB level of approximately 170 mg/L within 15 minutes. Blood GHB concentrations in the range156 – 260 mg/L induced moderately sound sleep.

CONCLUSIONS: This subject's elevated GHB concentration, significantly in excess of that expected from her prescribed dose, and her combined use of CNS depressant drugs, together with her problematic sleep apnea, and snoring (both contraindications for GHB use) were determined to have caused this subject's death. The manner of death was determined to be accidental.

Postmortem Analysis: Development of an Analytical Strategy to Determine a Large Panel of Drugs on a Small Sample Volume

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AIMS: An 18 years old male died after a heavy struggle with four policemen. The body presented multiple but not severe blunt trauma injuries (contusions, abrasions and few lacerations to the head), not able to cause the death. The case was classified as sudden death after a violent struggle, with a probable induction of hypoxia. Besides, depositions of the deceased night-mates agreed that he claimed to have assumed different drugs of abuse. We were assigned the responsibility to determine whether a massive drug assumption could have generated such a major respiratory failure or rather the hypoxic condition, eventually responsible for the death, resulted from constriction in handcuffed, face down position, and increased oxygen need upon the scuffle.

METHODS: Some months after the death we received tiny amounts of urine, blood and bile specimens. We were requested to screen for the presence of ketamine,

norketamine, amphetamines, heroin, 6-MAM, 11-nor-9-carboxy-Δ^9-THC and, possibly, anabolic steroids. Screening all these substances appeared problematic, since the blood volume available (0.5 mL) did not allow to perform different analytical procedures on separate aliquots. Furthermore, the specific gravity of the urine sample showed an anomalously low value, likely due to dilution during bladder washing, making this matrix unreliable for drug analysis. Both blood and bile specimens were processed using a specifically designed protocol of analysis. The samples were subjected to a preliminary enzymatic hydrolysis and a subsequent extraction step with tert-butyl methyl ether at pH 9. Then, the extracts were directly analyzed by GC-MS and HPLC-MS, using different selected ion monitoring (SIM) programs for ketamine, norketamine, amphetamines, morphine and 6-MAM. The extracts residues were subsequently derivatized with a mixture MSTFA-NH_4I-DTE and lastly re-analyzed by GC-MS, using different SIM methods to determine morphine, 6-MAM, THC-metabolites and various anabolic steroids. This protocol was utilized to build up 5-point calibration curves from spiked negative blood for all the analytes cited. Limits of detection, defined as the minimum concentration providing S/N > 3 ratio, were extrapolated from the lowest concentration level used for the calibration. All LODs resulted below 5 ng/mL.

RESULTS AND CONCLUSIONS: Both blood and bile samples turned out positive for morphine. Using the HPLC-MS/MS calibration, the morphine concentration in blood was 26 ng/mL and in the bile sample was 4800 ng/mL. The GC-MS determination found a morphine concentration of 20 ng/mL in blood and 2100 ng/mL in bile. By considering that i) the concentration of morphine in blood was low, ii) the concentration of morphine in bile was high and iii) the metabolite 6-MAM was absent, we concluded that the presence of morphine had to be attributed to an earlier use of heroin or morphine. With a long elapsed time between drug use and death a link between these two events is unlikely. Therefore, the forensic pathologist together with other autopsy evidence determined that the sudden death was due to a respiratory and heart failure related to “excited delirium syndrome”.

Fatal Oleandrin Poisoning of a Herd of Cattle

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CASE REPORT: Oleander (Nerium Oleander L.) is an ornamental shrub that grows in many parts of the world and Mediterranean countries including Cyprus. All parts of the plant are poisonous because of the presence of the cardiac glycoside oleandrin. The present study presents a case of mass fatalities in a heard of 100 cows with emphasis on the importance of toxicological analyses. The cows died with no specific suspicion of cause of death, originally. Feed from the farm was first analysed for pesticide residues with no indication of pesticide residues. Blood, urine, stomach contents and liver samples were collected for analysis. It was suggested later that it could be poisoning from oleander leaves contained in the feed. This was later confirmed through toxicological analyses and Veterinary Services and Police investigation. The owner of the farm subcontracted the feed preparation to a local company that mixed various leaves and shrubs in the feed. At that particular time, a worker in this company mixed leaves from oleander trees with a number of other leaves from various trees and bushes. This was consistent with a later report from veterinarians that animals died from dysrhythmia.

METHODS AND RESULTS: Immunoassay is a rapid and convenient method for screening of biological samples for the ingestion of N. Oleander. Because of its structural similarity, cross-reactivity has been demonstrated between the cardiac glycosides in N. Oleander and the digoxin immunoassay. Consequently serum and urine samples were assayed using the homogenous enzyme immunoassay (EMIT 2000) for digoxin related compounds. Serum pools were supplemented with oleandrin (Sigma Chemicals) and digoxin equivalents were detected at concentrations at or above 0.16ng/ml. Serum samples from the dead cows were found to have apparent digoxin concentrations from 0.38 - 2.8 ng/mL. Leaves from the feed were identified by microscopic examination of the epidermis where stoma cells are typical, and then isolated and assayed by TLC - after sample clean-up with Extrelut NT columns and extraction with a dioxane/methanol/dichloromethane mixture (8:1:1). Stomach contents were similarly screened by TLC in conjunction with an oleandrin standard. Following presumptive identification with immunoassay and TLC, oleandrin was confirmed by LC-MS/MS (triple quadrupole linear ion trap mass spectrometer) in serum, urine and liver. Method validation was carried by six replicate fortifications of serum and urine at 1ppb oleandrin and six replicate fortifications of liver at 5ppb oleandrin. Good recoveries were obtained from samples and oleandrin was confirmed to be present in the samples. The sensitivity and specificity of the LC-MS/MS analysis enables the method to be the method of choice for oleandrin poisoning, despite its complexity and high cost.

Antipsychotics Associated with Pulmonary Emboli in a Swedish Medico-Legal Autopsy Series

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AIMS: Patients suffering from psychotic diseases have a higher cardiovascular mortality rate. Moreover, treatment with antipsychotics has recently been associated with an increased risk of venous thromboembolism. The aim of this study is to determine the association between fatal pulmonary emboli and the use of antipsychotic drugs in a Swedish medico-legal autopsy series.

METHODS: Persons aged 18-65 years and subjected to a medico-legal autopsy in 1992 through 2005 were selected. Based on the external cause of death, determined by the forensic pathologist, unnatural deaths (including fatal intoxications) were excluded. Subjects were identified where pulmonary emboli was the cause of death. Use of antipsychotics was based on the results of the postmortem analyses and categorised as use of high-potency antipsychotics (flupenthixol, fluphenazine, haloperidol, perphenazine, pimozide, trifluoperazine, zuclopenthixol), low-potency antipsychotics (chlorpromazine, chlorprothixene, dixyrazine, levomepromazine, melperone, thioridazine), second generation antipsychotics (clozapine, olanzapine, risperidone, quetiapine, ziprasidone) or no use of antipsychotics. Logistic regression analyses were performed using non-users of antipsychotic drugs as reference group.

RESULTS: During the study period, 14,439 subjects were included; pulmonary embolism was recorded as the cause of death in 279 subjects. Use of antipsychotics was verified in 538 subjects and in 30 subjects a combination of different antipsychotic substances were used at the time of death. Among the subjects with pulmonary emboli, 33 were users of antipsychotics. Use of low-potency antipsychotics and second generation antipsychotics were significantly associated with fatal pulmonary emboli (adjusted OR: 2.39; 95% CI; 1.46 - 3.92 and 6.91; 95% CI; 3.95 - 12.10, respectively). Out of 26 subjects classified as high-potency antipsychotic drug users, none had pulmonary emboli as the cause of death.

CONCLUSIONS: Pulmonary emboli were overrepresented among medico-legal autopsy cases identified as users of low-potency and second generation antipsychotics.

Extraction of Fluoxetine and Norfluoxetine from Liver Samples using a New Mixed Mode SPE Sorbent

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AIMS: The aim of this poster presentation is to inform the forensic toxicology community of a new sorbent methodology for the extraction and analysis of fluoxetine and its primary metabolite from postmortem liver samples. The new sorbent is a mixed mode SPE column based on a polystyrene-divinyl benzene/carboxylic acid sorbent (SSCCXH).

METHODS: In this procedure, samples of bona-fide postmortem liver homogenates (0.1 to 0.5 g of 1:4 tissue homogenates) were spiked with fluoxetine and norfluoxetine (100 to 1000 ng) and IS (Proadifen, 100 ng). Samples were buffered with pH 6 phosphate buffer (9 mL), mixed and centrifuged. The supernatants were applied to the SPE columns, which had been previously conditioned with methanol and pH 6 buffer (3 mL, respectively). After application of samples, the sorbents were washed with pH 6 buffer, acetonitrile, and hexane (3 mL of each). The columns were then dried under full vacuum before being eluted with an ethyl acetate-methanol solution (2 x 3 mL of a 9:1 mix). To the eluate 0.1% methanolic hydrochloric acid was added (50 µL) before being evaporated to dryness. The residue was dissolved in 200 mL of deionized water for injection onto a liquid chromatograph (50 mL) using photo diode array detection (LC-PDA, 230 nm) for analysis using an isocratic mobile phase of acetonitrile : aqueous TFA (35:65). The same method for extracting fluoxetine and norfluoxetine from liver homogenates was applied to a silica based mixed mode SPE column with a propyl functionality/ carboxylic acid phase (CUCCX1) as a comparison.

RESULTS: In this presentation, the efficient metho–dology for the extraction of fluoxetine and norfluoxetine from liver homogenates using this new sorbent has shown to have the ability to detect 100 ng of these specific drugs from 0.5 g of 1:4 liver homogenates. The linearity of extraction is >0.99 over the range 100 to 1000 ng for the individual drugs, and the recovery is greater than 80% over the range of homogenate solutions (0.1 g to 0.5 g). Chromatograms comparing the cleanliness and sensitivity of the extraction are presented alongside a standard silica based SPE material.

CONCLUSIONS: Fluoxetine and norfluoxetine can be

simply and efficiently extracted from postmortem tissue samples using as little as 0.1 g of a 1:4 homogenate (0.025 g of the original tissue sample). Using this simple SPE procedure with LC-PDA will go a long way to assist toxicologists engaged in the analysis of drugs in this matrix.

Therapeutic Misadventures with Selective Serotonin Reuptake Inhibitors (SSRIs): Fatalities from Drug Interactions and Impaired Performance

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An increasing number of toxicology cases involve fatalities where an SSRI was co-administered with multiple medicinal agents such as narcotic analgesics or antitussives; anxiolytics; antidepressants, CNS stimulants or depressants; and illicit recreational drugs. Many of these medications (indicated by an*) are serotonergic in nature and the toxicity from increased serotonin (5-HT) has been designated as Serotonin Syndrome (SS). SSRIs may also inhibit cytochrome P450 enzyme activity, resulting in higher plasma drug concentrations (C-max) and/or a reduced time to maximum plasma concentration (T-max) for certain drugs. Fatal outcomes from these combinations of medicinal agents can be assigned to one of three categories: [1] fatal SS from high 5-HT concentration; [2] fatal drug interaction from metabolism inhibition; and [3] fatal accidents from drug-impaired performance. The following data illustrates cases from categories 2 and 3. Due to abstract space limitations a discussion on postmortem redistribution and pharamcogenomic differences is not included here.

CASE 1: Hospitalized male on long-term therapy with phenytoin, trazodone*, valproic acid, citalopram* (SSRI), olanzapine* and atropine. Pre- and post-surgery medications included meperidine*, hydromorphone, morphine, ondansetron*. The subject found unresponsive about 34 hours after surgery. Examination of the PCA pump and the pharmacy records showed no discrepancy in pump performance or meperidine dose preparation (4-hour limit of 150 mg). Postmortem toxicology (blood) results were: meperidine 1908 ng/mL; normeperidine 1590 ng/mL; citalopram 194 ng/mL. The expected C-max for citalopram would be in the range of 50 - 100 ng/ml and the expected C-max for meperidine would be in the range of 200 - 500 ng/mL.

CASE 2: Hospitalized female subject on long-term therapy with clonazepam, tramadol*, escitalopram* (SSRI) and cyclobenzaprine. Pre- and post-surgery medications included ondansetron*, morphine, and meperidine*. The subject was found unresponsive about 19 hours after surgery. Examination of the PCA pump and the pharmacy records showed no discrepancy in pump performance or meperidine dose preparation (4-hour limit of 150 mg). Postmortem toxicology (blood) results were: meperidine 915 ng/mL; normeperidine 795 ng/mL; morphine trace; tramadol 249 ng/mL; and citalopram 480 ng/mL.

CASE 3: Non-hospitalized post surgery male subject on long-term therapy with morphine (60 mg/day), hydrocodone, sertraline* (SSRI), lithium*, amphetamine*, codeine and diazepam. Postmortem toxicology (blood) results were: tramadol* 520 ng/mL; morphine (total) 1100 ng/mL; morphine (free) 150 ng/mL; sertraline 200 ng/mL and dextromethorphan* 370 ng/mL. The expected C-max for sertraline would be in the range of 20 - 300 ng/mL and the expected C-max for a 20 – 100 mg dose of dextromethorphan would be in the range of 5 - 200 ng/mL.

CASE 4: Non-hospitalized female subject on long-term therapy with escitalopram* (SSRI) and bupropion*. The subject allegedly took 20 mg zolpidem as a sleep aid prior to 2400 hours. She sustained relatively minor injuries in a driving accident that resulted in a fatality of another motorist at 0745. Blood collected at 0915 was found to contain bupropion 379 ng/mL, citalopram 63 ng/mL and zolpidem 171 ng/mL. The expected zolpidem concentration after about 10 hours would be in the range of 10 - 30 ng/mL.

CONCLUSIONS: This data suggests a high risk for toxicity from a variety of medicinal agents co-administered with SSRIs.

Methadone Related Deaths in Norway 2004-2006

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AIMS: Methadone, a potent opioid receptor agonist, is the most commonly used drug in the treatment of opioid dependence in Norway. Methadone's side-effects and toxicity are well documented, and the potential for a rise in the incidence of methadone related deaths due to greater methadone availability is worrying. This study aims to follow the occurrence and pattern of methadone related deaths over a three-year period. We will also examine cases where methadone was present at toxicological analysis postmortem and assess what role methadone toxicity played in the cause of death.

METHODS: Toxicological results where methadone was positive were collected from a database of forensic autopsies at the Division of Forensic Toxicology and Drug Abuse (DFTDA). Cases were divided into those where methadone was the only drug present and those where other drugs were present in addition to methadone. All cases were assessed individually.

RESULTS: A total of 176 methadone related deaths were identified from our database of approximately 5,400 cases for the period 2004 through 2006. Of these, 143 were male and 33 female. Methadone was the only

finding at toxicological analysis in just 6 (3.4%) of these cases. Methadone concentrations in blood varied from 0.06 mg/L to 4.64 mg/L, with a median concentration of 0.43 mg/L. A variety of other substances were also detected in blood, the 3 most common being tetrahydrocannabinol (THC, the primary psychoactive constituent of cannabis), and metabolic products of the benzodiazepines diazepam (N-desmethyldiazepam) and clonazepam (7-aminoclonazepam), respectively. Morphine was detected in 35 cases. The mean number of additional substances detected in these cases was 4.

CONCLUSIONS: Methadone related deaths in Norway have risen over the past years. Methadone was most often detected in combination with other substances. These results may suggest that patients using methadone have a significant use of other substances, both illegal and prescription medicines. Alternatively, results may indicate that the majority of those involved in methadone related deaths are not enrolled in rehabilitation programs, and that the source of methadone in these cases is diversion. Further analysis of the results is necessary and we are in the process of coupling our material to the National cause of death register and regional registers of patients enrolled in rehabilitation programs (LAR).

The New 'Ice' (Crystal Methylamphetamine) Age?

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AIMS: "Ice" is a street name for crystal methy–lamphetamine hydrochloride, which is a powerful, central nervous system (CNS) stimulant drug. "Ice" is more potent than other forms of amphetamines due to the fact that it is much purer than the powder form of methylamphetamine hydrochloride (methamphetamine or 'speed') currently available on the 'street'. It looks like delicate, benign flakes of ice for which it is named, but this substance is an insidious, sometimes fatal, drug which has worked its way into Australia's drug culture with devastating effects. 'Ice' usage has increased quite markedly in Australia – and has been detected in an increasing number of driving under the influence (DUI) cases. The aim of this study is to investigate the usage and impact of methylamphetamine and the purer form, methylamphetamine hydrochloride or 'ice,' on traffic safety.

METHODS: In this study, 585 drug positives (DUIs) of drivers were drawn from our files for the year 2005 and examined for the presence of methylamphetamine. These were compared to an earlier period of cases drawn from 1990. The blood samples taken from the drivers were analysed at the Division of Analytical Laboratories (DAL), Lidcome, New South Wales, Australia. Police at the roadside, using a field assessment checklist, carried out assessments for visible signs of possible intoxication.

RESULTS: Of the 585 drug positives received in 2005, 200 were positive for methylamphetamine detections; of these 5 admitted to consuming 'ice' and 16 were long distance heavy vehicle drivers. These results were compared to only 237 drug positives received in 1990 of which 51were stimulant drugs that included drugs such as ephedrine as well as amphetamines. This represents an increased positive drug detection of almost two and a half times (2.47) over fifteen years, with an increase in use of stimulants (including methylamphetamine) from 21.5% in 1990 to 34.2% (methylamphetamine usage) in 2005. Crystal methylamphetamine or 'ice' has a range of nicknames such as 'shabu', 'crystal meth', 'glass', but have the same effect. About 73,000 people are now believed to be 'hooked' (physically dependent) on the drug which was mainly used (and still is) used by drivers of heavy vehicles ('truckers') to keep awake on their long-distance drives. A case study is presented (which was included in the figures previously for 2005) where a young male ingested a large amount of 'ice' while driving a motor vehicle. He was observed by police driving his motor vehicle on the wrong side of the highway at 115 kph. He was eventually pulled over by police, and he got out of the car and walked to the back of the vehicle holding a cigarette packet. Police saw he was crunching something hard and had a white crystalline substance around his lips. He was told to spit it out but he failed to comply. His teeth appeared clenched together and he appeared very tense. He then started 'fitting', his body went tense, his legs thrashing about and he appeared to lose consciousness, then he stopped breathing. Cardiac pulmonary resuscitation (CPR) was commenced until ambulance officers arrived. Resuscitation attempts were unsuccessful. A white crystalline substance was found in the motor vehicle which was identified as crystalline methylamphetamine hydrochloride which had a purity of 75%. A femoral blood sample taken from the deceased was found to have a massive methylamphetamine concentration of 80.75 mg/L.

CONCLUSIONS: Our findings indicate that there has been a marked increase in the use of 'ice' or crystal methylamphetamine by drivers of motor vehicles with dangerous consequences to themselves and other road users.

Asphyxial Death Utilizing a Commercial Propellant Duster: 1, 1-difluoroethane

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CASE REPORT: A 22-year-old man with previous suicide attempts was discovered deceased in a prone position in bed. A black plastic refuse bag was secured over his head with a waist belt about the neck. Inside

the bag were a can of "Dust Off" dust remover and approximately 100 mL of green liquid. An empty bottle of Nyquil was on the nightstand. "Dust-Off" contains 1,1-difluoroethane (DFE) which is a colorless, odorless gas is utilized commercially as a propellant in a myriad of consumer products including consumer hygiene products, refrigerants, and computer keyboard cleaners. The intoxicating effects and accessibility of products containing DFE are appealing to inhalant abusers. Clinical effects associated with exposure range from minor upper respiratory irritation to serious sequelae including ventricular dysrhythmia and pulmonary edema. Accidental deaths have been associated with misuse.

METHODS AND RESULTS: Postmortem Evaluation: The external examination was unremarkable. Internal examination was notable for severe pulmonary congestion and severe cerebral edema (1641 grams). The tracheal and bronchial mucosa was erythematous and coated with frothy, clear, mucoid material. The mucosal surface of the esophagus was coated with green liquid material. The stomach was devoid of gastric contents. Histological sections of lung revealed marked septal capillary congestion and regions of edema. The remaining organs were grossly and microscopically unremarkable.

TOXICOLOGICAL ANALYSIS: Preliminary analyses indicated the presence of doxylamine, dextromethorphan, and acetaminophen. These drugs were quantitated in femoral blood. Additionally, blood, urine and vitreous were analyzed by GC headspace for ethanol. During autopsy lung tissue and blood were collected in 10 mL headspace vials and sealed with Teflon caps. The vials were refrigerated and analyzed within days. The toxicology results were as follows: Ethanol: blood, 0.14 g/dL; urine, 0.05 g/dL; vitreous, 0.10 g/dL; Acetaminophen: blood, Positive; Dextromethorphan: blood, Positive; Doxylamine: blood, Positive. DFE: blood, Positive; lung, Positive.

CONCLUSIONS: Asphyxia resulted from inhalation of this DFE containing product. The presence of severe cerebral edema and pulmonary congestion are consistent with a delayed death. Scene investigation and autopsy findings were consistent with a suicidal manner. The low blood levels of Nyquil constituent drugs and discovery of Nyquil emesis within the plastic hood are consistent with ingestion near death, a backup planned to prevent failure of the asphyxial method. The popularity of inhalant abuse using commercially available products is reportedly increasing. Accidental and suicidal deaths are expected to parallel this trend.

Concurrent Appearance of Vanoxerine, mCPP and 'Wormazine' on the Belgian Market: A Case Study

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AIMS: Vanoxerine (GBR-12909) is a high-affinity dopamine reuptake inhibitor and is currently undergoing clinical trials as a possible medication for the treatment of cocaine addiction. To the best of our knowledge, vanoxerine has not yet been encountered on the Belgian market in co-appearance with illicit drugs. In contrast, mCPP or meta-chlorophenylpiperazine is increasingly seen in powders and tablets that are being sold as ecstasy. The effects of mCPP are quite variable, but most users describe an ecstasy-like effect. Several side effects are also reported, among which nausea, dizziness, hallucinations and headache. In addition, a serotonin-like intoxication syndrome has been described, linking mCPP to one of the hepatic metabolites of the antidepressant trazodone (Desyrel®). We present here a case study that describes the concurrent appearance of vanoxerine, mCPP and 'wormazine' in seized powders and tablets on the Belgian market.

METHODS: In this case study, GC-MS and NMR techniques were used to determine the identity of the active component(s) of seized powders and tablets. Mass spectrometric analyses were conducted with an Agilent 6890N GC coupled to an Agilent 5973N MSD operating in EI-mode (70 eV). Chromatographic separation was achieved via a Varian VF5-MS factorFour capillary column (30 m x 0.25 mm x 0.25 µm) with He as carrier gas (1 mL min-1) and applying a multiple-step linear temperature program: 50°C (hold 1 min), ramp to 100°C at 35°C min-1 (hold 0 min), increase to 310°C at 10°C min-1 (hold 30 min).

RESULTS AND CONCLUSIONS: Both methods confirmed the first illegal appearance of vanoxerine in Belgium. The co-appearance of vanoxerine with mCPP and the antihelminthic 'wormazine' attracted our attention. 'Wormazine' is nothing else than the hydrochloride salt of piperazine and can be viewed not only as a precursor for the synthesis of vanoxerine and mCPP, but also other piperazine derivatives with psychotropic activity linked to recreational drugs like 1-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)-piperazine (TFMPP) and 1-(4-methoxyphenyl)-piperazine (MeOPP). Hence, the appearance of piperazine may be suggestive of a possible surreptitious synthesis pathway.

Lethal Poisoning from Yew Tree Needles

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CASE REPORT: The yew tree (Taxus baccata L.) is widely spread all over central and southern Europe. The toxicity of yew has been well known. An 18-year-old woman drank up a decoction of taxus needles from small yew tree in a flowerpot which she bought in a garden-tillage. About 2 hours after her father found her in a comatose state. About 6 hours after drinking of the decoction she died in a hospital. The autopsy findings were nonspecific: hyperemia of the lungs, liver, kidney, spleen, cerebral oedema. In the stomach there were found a few needles on the stomach wall.

METHODS: About 20 mL of gastric content, 20 mL of urine and 5 g of liver were worked up for determination of yew matters. Crude taxine extract as a reference solution was prepared as described by Theodoritis et al. [1] (1 g of tree needles was ground and then extracted with about 50 mL of methanol. The methanolic macerate was evaporated to dryness and the residue was reconstituted with 1 mL of methanol, 0.1 mL of which was applied to a SPEC-C18 (Varian): condition - 1 mL of methanol, 1 mL of deionized water; load sample; rinse - 2 x 1 mL of deionized water, 1 mL of 20% methanol in water, 1 mL of 50% methanol in water; 5 min. vacuum; elute - 2 mL of methanol. The eluate was evaporated to dryness and reconstituted with 0.5 mL of acetonitrile). The same procedure was used for extraction of gastric content. 5 g of liver were homogenized and precipitated with amoniumsulphate in acid pH and then extracted on SPE (Evidex, 600 mg): condition - 6 mL of methanol, 6 mL of phosphate buffer (pH 6); load sample (pH 6); rinse - 6 mL of deionized water, vacuum (1 min.), 1 mL of n-hexane, vacuum (5 min.); elute - 3 mL of ethyl acetate-methanol (98:2) and 2 x 3 mL of dichlormethane-isopropanol-ammonium hydroxide (4:1:0,1). Combined eluates were evaporated to dryness and reconstituted with 0.2 mL of acetonitrile. Urine was extracted with diethylether after acidification and alkalization. Chromatographic separation was achieved by HPLC on a Gemini-C18 column (150 x 2 mm; Phenomenex). MS detection was performed on a single quadrupole with ESI source in positive mode (LC-MS-2010A, Shimadzu). The mobile phase was pre-mixed 0.01 M ammonium acetate buffer with 0.1% formic acid (MFA) and acetonitrile (MFB) used in a gradient mode. A gradient starting with 15% MFB was increased linearly to 90% MFB over 25 minutes, held for 10 min., then decreased to 15% MFB. The flow rate was 0.2 mL/min. The full scan mode in the range m/z 200 - 900. Analysis was focused on determination of taxine B and isotaxine B with m/z = 583 (protonated moleculer ion - 584) and other pseodo-alkaloids of ”taxine fraction” [2,3] (with m/z: 541, 567 (2x), 583 (2x), 625 (2x), 651, 667).

RESULTS: The chromatograms of stomach content, liver and urine extracts were compared with the chromatogram of crude taxine extract. In the stomach content all of taxine pseudo-alkaloids of "taxine fraction" were determined. In liver and urine extracts were found two substances with $[M+H]^+ = 584$, with the same retention time as substance in crude taxine and stomach content extracts.

CONCLUSIONS: Based on the history and toxicology findings, the case was classified as a suicide.

References:

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Determination of Lead in Biological Specimens From a Homicidal Poisoning Case with Kohl (Lead Sulfide)

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CASE HISTORY: Kohl is a grey or black powder used as an eye cosmetic in Middle East, India, Pakistan and some parts of Africa. In Upper Egypt Kohl is considered as one of the most common and cheap traditional eye cosmetics. To date, postmortem concentrations of lead from kohl poisoning were not recorded. We report a case of unusual homicidal poisoning by Kohl.

A 20-year-old female got pregnant illegally. Her mother prepared her an omelet sandwich mixed with kohl. The same day she was admitted to the hospital and after one hour she died. An autopsy was performed. Samples were sent for toxicological analysis. The aim of this research is to determine the lead in postmortem human biological specimens using Flame Atomic Absorption Spectrophotometry (FAAS).

METHODS: Stomach contents, blood, liver and kidney were received by Assuit Chemical Laboratory of the medico-legal department. Screening of sulfide was performed by adding a solution of 3 mL of dilute sulfuric acid to 10 gm of the stomach contents. Hydrogen sulfide fumes turned lead acetate paper black. Lead was determined by using 10 gm of the tissue or 10 mL of the blood. The tissue samples were segmented into small pieces and homogenized in a blender. The samples were digested with 20 mL of concentrated HCl (12 N) for 30 minutes in a water bath. The resulting digest was filtered and diluted with 50 mL deionized water. FAAS was used to determine the lead concentration.

RESULTS AND CONCLUSIONS: Analysis of postmortem specimens revealed the following lead concentrations: blood (33.41 mg/L), liver (13.48 mg/Kg) and kidney (12.72 mg/Kg).

Analysis of Psychotropic Drugs in Human Body Fluids Using Disk Solid-Phase Extraction

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AIMS: Disk solid phase extraction (SPE) technology has been extensively introduced as a second generation SPE. Disk SPE cartridges consist of a thin membrane disk containing chromatographic sorbent particles mounted in the bottom of polypropylene syringe barrels. Many benefits of disk SPE methods over conventional SPE techniques for sample preparation have been reported. The membrane disks are composed of polytetrafluoroethylene or glass fiber impregnated with fine bonded silica, which should reduce the channeling and clogging sometimes associated with viscous biological matrices, such as plasma or serum, in conventional SPE. Moreover, the small bed volume and sorbent mass within the disk SPE cartridges, compared to the conventional SPE, allow for the use of a reduced solvent volume, smaller elution volume, reduced time for the evaporation step and higher throughput. In this presentation, we show a simple, rapid, and simultaneous determination of psychotropic drugs in human plasma and urine samples using disk SPE followed by high-performance liquid chromatography (HPLC).

METHODS: Human plasma and urine (1 mL each) containing seven phenothiazine drugs, perazine, perphenazine, prochlorperazine, propericiazine, thioproperazine, trifluoperazine, and flupentixol, were mixed with 2 mL of 0.1 M NaOH and 7 mL of distilled water, and then poured into Empore C_{18} disk cartridges. Analytes were eluted with 1 mL of chloroform-acetonitrile (8:2), and detected by HPLC with ultraviolet detection.

RESULTS: All drugs were well separated from each other and produced sharp peaks under our HPLC conditions. While small impurities were observed for both plasma and urine blanks, no interfering peaks were found around the peaks of the analytes of interest. Recoveries of the seven phenothiazines spiked into plasma and urine samples were 64.0 - 89.9% and 65.1 - 92.1%, respectively. Regression equations showed excellent linearity (r^2 = 0.9872 - 0.9981), with detection limits of 0.021 - 0.30 µg/mL for plasma and 0.017 - 0.30 µg/mL for urine. The intra- and inter-day coefficients of variation for both samples were commonly below 9.0% and 14.9%, respectively.

CONCLUSIONS: In view of its simplicity and quantitative nature, the present method is recommended for the determination of phenothiazaine drugs in human body fluids at high concentrations in forensic toxicology and clinical toxicology.

Methylenedioxymethamphetamine (MDMA)-Related Deaths in Taiwan: 2001-2005

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AIMS: Methylenedioxymethamphetamine (MDMA) has gained renewed popularity in the "club" scene in Taiwan. To access the epidemiology and causes of death of MDMA-associated fatalities, all 42 cases that were tested positive for MDMA between January 2001 and December 2005 were reviewed.

METHODS: All MDMA-positive cases were initially identified by TDx or AxSYM Fluorescence Polarization Immunoassay for Amphetamines adapting 300 ng/mL as the cutoff. MDMA in these specimens were confirmed and quantitated by gas chromatography-mass spectrometry (GC-MS). All samples were also screened for basic and acidic drugs by GC-MS, and then quantitatively analyzed by GC-MS. Ethanol concentrations were determined in biological fluid samples by headspace gas chromatography with flame ionization detector.

RESULTS: The distribution of these 42 cases during the 5-year (2001–2005) period was 3, 7, 9, 14 and 9, respectively. The mean age of these MDMA-related fatalities was 24.6, ranging from 15 to 46, while 25 (59.5%) of these deaths were men. Of these MDMA-positive fatalities, the cause of death for 32 were ruled as acute drug (MDMA) intoxication, 8 mechanical injury (blunt trauma, gunshot, falling), 1 hanging, and 1 drowning. For the manner of death, the numbers of cases ruled as accidental, homicidal, suicidal and undetermined were 31 (73.9%), 5 (11.9%), 3 (7.1%) and 3 (7.1%), respectively. Among the 32 cases in which MDMA was ruled as the cause of death, the concentration of MDMA in a substantial number of blood samples fall in the recreational use range (0.12–40.41 µg/mL) with a mean value of 4.76 µg/mL. MDA was found in 23 of these cases ranging from 0.050 to 1.81 µg/mL (mean 0.220 µg/mL). Of these 32 deaths, blood level of multiple drugs was found in 17 cases; MDMA was the only drug found in 15 cases; while the numbers of cases containing ketamine, heroin, benzodiazepines, ethanol, lidocaine, methamphetamine and phencyclidine were 10, 4, 4, 4,

3, 2 and 1, respectively. Blood MDMA concentrations in the other 10 MDMA-positive cases ranged from 0.084 to 11.48 µg/mL (mean 2.167 µg/mL); MDA was found in 5 of these cases ranging from 0.063 to 2.350 µg/mL (mean 0.647 µg/mL); while the numbers of cases containing ketamine, benzodiazepines, methamphetamine and ethanol were 6, 4, 3 and 2, respectively.

CONCLUSIONS: MDMA showed a wide range of concentrations. Higher concentration was seen in cases where death was attributed to the effects of MDMA alone. MDMA concentration overlaps considerably among cases in which MDMA was ruled as the cause of deaths and in cases where death was caused by trauma.

Suicide Attempt of a Pregnant Woman by Clozapine Ingestion in Late-Term Pregnancy Resulting in a Fatal Neonatal Intoxication

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AIMS: This report concerns a fatal poisoning of a neonate during the final stage of gestational life evoked by his mother, who, while 9 months pregnant, took a toxic dose of clozapine in an attempt to commit suicide. She was also severely poisoned, but ultimately was saved. The purpose of the study is the presentation of the toxicological findings in specimens collected from the neonate and the interpretation of the results which apart from toxicological aspects – also involves issues associated with psychiatry, pharmacotherapy in pregnancy and medico-legal problems.

METHODS: The autopsy blood and homogenized liver and kidney samples were subjected to liquid/liquid extraction with the use of ethyl acetate. The biological extracts were analyzed by LC-MS method using a Finnigan MAT LC in a gradient mode coupled to a LCQ mass spectrometer equipped with an ion trap APCI source.

RESULTS: The toxicological findings in the neonatal specimens were:

	Clozapine	Norclozapine	Clozapine-N-oxide
Specimens	Concentration (µg/mL), (µg/g)		
Blood	7.3	2.6	0.5
Liver	28.0	17.1	31.1
Kidney	10.1	6.1	5.8

CONCLUSIONS: The toxicological findings obtained may explain the death of the neonate. In the analyzed case, the attending psychiatrist decided to withdraw clozapine from the treatment regimen at the beginning of pregnancy; however, it was probably safer to continue clozapine in pregnancy than to stop it. Clozapine treatment in and of itself does not ensure freedom from suicide attempts.

Postmortem Sevoflurane and Midazolam Distributions After Supposed Intravenous Self-Administration

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CASE REPORT: We report a case of a 35 year old woman found unconscious in a hotel room, breathing spontaneously with bilateral non-reactive mydriasis. Fourteen 5 mg midazolam vials, several empty 10 mL syringes and a bottle of Ultane® (sevoflurane, volatile liquid aesthetic) were found near the body. No mask, gauze pad or bag was present in the room. Blood and urine were collected at hospital admission. A brain CT scan showed total destruction of thalamic central nuclei. Death was then declared but the victim was maintained under artificial ventilation for 4 days. During autopsy, additional biological specimens were collected.

METHODS: Analyses were performed by GC-FID (ethanol), HPLC-DAD (general unknown screening), headspace-GC/MS (sevoflurane) and LC-MS/MS (sedatives). As no sevoflurane was detected, its metabolite, hexafluoroisopropanol (HFIP) was assayed using HS-GC/MS (characteristic ions (m/z): 99, 129 and 79). HFIP represents about 5% of sevoflurane concentrations. Using the Imbriani formula (1), it is possible to estimate sevoflurane air concentrations in case of inhalation.

RESULTS: The findings are summarized in the table below.

Sample	HFIP (mg/L or mg/Kg)	Sevoflurane, estimated (mg/L or mg/Kg)	Midazolam (ng/mL or ng/g)	OH-Midazolam (ng/mL or ng/g)	Zolpidem (ng/mL or ng/g)
Peripheral blood (admission)	21.01	420	60	12	14
Urine (admission)	597.5	11950	51	9901	11
Urine (autopsy)	2.36	47.2			
Heart blood	1.2	24	0.2	1.1	
Vitreous	0.53	10.6	0.4	1.1	
Liver	3.72	74.4	0.6	33.1	
Bile	3.37		4.1	145.5	
Brain	1.11		44.4	8.6	
Lung	3.36				
Fat (epiploon)	0.86				
Heart			0.4	1.5	
Hair			81	Trace amounts	2535

Using (1), Avogadro gas law and according to room volume (43 m^3), the decedent should have inhaled 36 Ultane® bottles to obtain such HFIP urinary concentrations. The suspected route of sevoflurane administration was intravenous infusion, and the cause of death should be attributed to destruction of central nuclei. Evidently, no cardiopulmonary arrest occurred and midazolam does not appear to be a factor in the cause of death. At admission, it appears that midazolam and sevoflurane were already largely distributed in the body. Midazolam is selectively distributed in the brain as concentrations at Day 4 were still elevated. OH-midazolam is not incorporated in hair and these analyses demonstrated that the victim had already consumed midazolam and significant amounts of zolpidem before the event.

Reference:

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Identification of Sinicuichi Alkaloids in Human Blood and Urine After Intoxication Caused by Oral Intake of a Heimia Salicifolia Extract

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CASE REPORT: A 26 year old male came to the hospital around midnight complaining about muscle pain of the extremities and the tongue and slightly raised temperature. In the evening and during the night nausea, headache, singular vomitus and ague occurred. C-reactive protein and leukocyte levels were at normal range while creatinine kinase was increased. The patient reported the intake of an unknown amount of sinicuichi tea that had been fermented over 24 h by adding yeast and sugar. The patient was treated with dimenhydrinate (Vomex A®) and released from the hospital the following afternoon. Dried plant material of Heimia salicifolia, a species of the lythraceae family basically found in Central and South America, is sold as "sinicuichi" by several internet shops. Brewed up or fermented and consumed, the so called sinicuichi tea causes exhilarating feelings and an alteration of awareness accompanied by bradycardia, relaxation of the muscles and a pleasant faintness. Some species of the lythraceae family contain a number of biphenyl quinolizidine lactone alkaloids. Their pharmacological properties are not yet fully known. Vertine (cryogenine), the alkaloid showing the highest concentration in the plant material, is considered to be the primary source of the effects of traditional heimia salicifolia brew like anticholinergic, anti-inflammatory, sedative, tranquilizing and spasmolytic activity. Sinicuichi brew and heimia leaves are widely used for medication by the natives of Central and South America.

METHODS: A blood and urine sample taken shortly after submission and the plant material used were available for analysis. Applying the routine urine and blood screening methods (GC-MS and HPLC-DAD) did not lead to the identification of exogenous substances. After liquid extraction with acetone five different alkaloids were detected in the plant material by our standard LC-MS screening method. This first analysis was carried out with an LC–MS/MS system operated in the Q1 scan mode applying a TurboIonSpray source. Chromatographic separation was achieved using a Synergi Polar RP column applying a gradient elution with a total flow of 0.25 mL/min and a runtime of 30 min. The information of the acquired spectra was used to set up a multiple reaction monitoring method (MRM) using two transitions per analyte. For MRM screening the chromatographic gradient was shortened to 15 min.

RESULTS AND CONCLUSIONS: Applying this MRM method to the patient's blood sample after liquid-liquid extraction, two of the five heimia alkaloids were detected qualitatively in the extract confirming the ingestion.

Target Analysis of GHB, GBL and 1.4-BD in Urine and Whole Blood by LC-MS/MS and Application to a Forensic Case

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AIMS: To develop a simple LC-MS/MS application for identification, confirmation and quantification of gamma-hydroxybutyrate (GHB), gamma-butyrolactone (GBL) and 1.4-butandiol (1.4-BD) in forensic cases.

METHODS: A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed for determination of gamma-hydroxybutyrate and its analogs in urine and whole blood. The extraction procedure involves a simple protein precipitation of 0.2 g whole blood spiked with internal standards (D6-GHB & D6-GBL). After mixture and centrifugation 100 µL of the supernatant is removed and diluted 1:1 with acidic water and injected. The urine sample (20 µL) is diluted with a solution of internal standards and injected directly into the LC-MS/MS system. A LC system (HP 1100 system, Agilent tech.) containing a Zorbax SB C18 column (150 x 2.1 mm, 3.5 µm) and a mobile phase of 10% methanol in water with 0.1% formic acid is used to separate the analytes within 10 min. Detection is performed by positive electrospray ionisation with a tandem mass spectrometer (Quattro micro, Waters) operating in multiple reaction monitoring (MRM) mode. In order to comply with EU guidelines the monitoring is

performed with two transitions of each analyte in order to gain a secondary identification parameter. This is achieved for GHB with sufficient sensitivity by making use of the in-source transformation of GHB to GBL.

RESULTS: The calibration curves of extracted whole blood standards are linear over a working range from 0.1 to 250 mg/Kg depending on the analyte, while in urine samples the linear range is from 1 - 500 mg/L. Preliminary results from the validation show a limit of detection (LOD) and quantification (LOQ) in whole blood ranging from 0.2 - 1 mg/Kg and 1 - 5 mg/Kg, respectively depending on the analyte. The intermediate precisions have relative standard deviations (RSD) below 20%, while the accuracy expressed by % bias is less than 15% depending on the analyte.

CONCLUSIONS: This simple, sensitive and reproducible method proved to be suitable for the determination of GHB and analogs in forensic cases. The method is applied in investigations of suspicion of drug rape and/or intoxication.

Analytical toxicology methods
Méthodes en toxicologie analytique

Screening for 145 Drugs in Blood Using Liquid Chromatography Tandem Mass Spectrometry

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AIMS: This paper describes development, optimization and validation of a method for the simultaneous screening of 145 multi-class drugs using liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) in positive mode. Our own library of mass spectral data and retention time was built up for general screening. Target compounds include drugs of abuse (opiates, cocaine, amphetamines, cannabinoids), antidepressants, benzodiazepines, hypnotics, neuroleptics, pesticides (organophosphorus, carbamates), antiepileptics, β-blockers, and some clinical drugs.

METHODS: d4-ketamine (internal standard) and 2 mL of phosphate buffer (pH 9.2) were added to blood which was extracted using a liquid–liquid (ether) extraction. The mixture was vortex-mixed for 1 min, and then centrifuged at 2500 rpm for 3 min. The organic layer was transferred into a 5 mL glass tube and evaporated to dryness at 55°C. The residue was reconstituted in 100 µL of mobile phase and 5 µL was injected into the LC-MS/MS system. Chromatographic separation was achieved with a Allure PFP Propyl column (100 x 2.1 mm, 5µ mm) and a phenomenex guard column. Data acquisition under MS/MS was achieved by applying multiple reaction monitoring (MRM) of two fragment ion transitions to provide a high degree of sensitivity and selectivity for both quantification and confirmation. The analysis time has been shortened in a 14 min run.

RESULTS: The decision on whether or not to report a sample as a positive result was judged according to the WADA criteria for qualitative assays based upon both the presence of a MRM response (signal-to-noise greater than three-to-one) within the retention time range (± 0.4 minutes) and a qualitative match between the relative intensities obtained and the corresponding reference standard (relative abundance less than 5%). The limits of detection for the majority of the compounds (107 in 145) were less than 1 ng/mL. Validation of the method was also established with 7 selected target compounds (MDMA, methadone, clozapine, ketamine, carbamazepine, haloperidol, codeine). The calibration curves of 7 drugs were excellent with correlation coefficients between 0.9919 and 0.9986, RSD were between 3.10% and 9.76%, and the low detection limits ranged from 0.1 to 10 ng/mg.

CONCLUSIONS: This method was successfully applied to the analysis of body fluids from forensic and clinical cases. The method we developed has the advantage of screening 145 drugs in one single extraction and detection system. The method was sufficiently selective, sensitive, and rapid to detect the drugs down to therapeutic concentration levels and is therefore feasible in forensic and clinical toxicology. The method can be applied to other biological samples and the number of target analytes can be increased.

Rapid Simultaneous Determination Method for Organophosphorus Pesticides in Human Serum by LC-APCI-MS

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AIMS: A simple and rapid method was developed for measuring 10 organophosphorus pesticides (Table 1) in serum by LC-MS. A case example is presented.

METHODS: Serum samples were deproteinized by acetonitrile and injected onto a C_{18} column using 10 mM ammonium formate-methanol as the mobile phase. The stability of organophosphates in serum was evaluated in fortified samples after storage at room temperature for 24 h, 4°C for 7d, and –30°C for 4 weeks and by subjecting samples to three freeze–thaw cycles. All studies were performed at three 0.625, 2.5 and 7.5 µg/mL.

RESULTS: Extraction recoveries were satisfactory and ranged between 60.0% and 119.3% in serum. Validation data (Table 1) demonstrated excellent linearity. Intra- and inter-assay accuracy and precision was evaluated

at three concentrations (0.625, 2.5 and 7.5 µg/mL) with satisfactory results. Table 1 data shows these results at 2.5 µg/mL.

Compound	LOD	LOQ	Linearity	R^2	Precision (CV%)		Accuracy (RE%)	
	(µg/mL)	(µg/mL)	(µg/mL)		Intra-assay	Inter-assay	Intra-assay	Inter-assay
acephate	0.25	0.375	0.375–8	0.9838	11.4	15	-13.8	-6.2
methidathion	0.5	0.625	0.625–8	0.9892	10.5	11.7	3.3	3.8
dichlorvos	0.5	0.625	0.625–8	0.9972	13.9	10.3	5	6.6
fenthion	1	1.25	1.25–8	0.9941	13	13	2.2	5
EPN	0.375	0.5	0.5–8	0.9904	13.7	11	-0.3	-1.5
diazinon	0.125	0.25	0.25–8	0.9967	6.9	5.4	2.8	4.8
phenthoate	0.25	0.375	0.375–8	0.9981	5.7	8.6	0.08	2
malathion	0.25	0.375	0.375–8	0.9991	8	11.1	8.6	-0.9
fenitrothion	0.125	0.25	0.25–8	0.9978	9.5	8.8	-4.1	4.5
cyanophos	0.125	0.25	0.25–8	0.9969	9.6	9	1.6	8.1

Stability studies demonstrated that dichlorvos and malathion exhibited the most rapid degradations over 24 h at room temperature. Methidathion and diazinon remained relatively stable during the entire 4 weeks testing period at all temperatures. The present method was successfully applied to one actual case of acute poisoning. In a 43-year-old male ingesting approximately 100 mL of both 5% fenitrothion and acephate emulsion, the determined serum concentrations of acephate and fenitrothion were 7.2 and 4.5 µg/mL, respectively.

CONCLUSIONS: This method is simple, accurate, and useful for the determination of organophosphorus pesticides and should be of benefit to both clinical and forensic toxicology.

Optimization of Opiate Detection by LC-MS

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AIMS: The aim of this study was to develop a selective and sensitive LC-MS method which is optimized for the simultaneous detection of multiple opiates. The following commonly encountered opiates were included: morphine, codeine, 6-acetylmorphine and dihydrocodeine.

METHODS: Thirty three different sets of conditions were evaluated during the method development and from these the best analytical conditions were selected to give the final method. Particular emphasis was given to optimization of the signal-to-noise (S/N) ratios and to maximising the intensities of parent and fragment ions. The final method was then validated using standards extracted from whole blood to determine its performance characteristics.
LC-MS analysis was carried out using a Shimadzu Model 2010 instrument fitted with a Merck LichroCART® column (125 x 2 mm) and guard column (10 x 2 mm) packed with Superspher® 60 RP-Select B stationary phase. The isocratic eluent composition was A:B = 85:15 (v/v) (A: 5 mM NH4COOH (pH = 3), B: acetonitrile: formic acid = 999:1 (v/v)) and was at a flow rate of 0.3 mL/min. The mass spectrometer was operated in APCI positive mode, with an ion source temperature of 250oC, CDL temperature of 230oC, block temperature of 200oC, N2 flow rate of 2.5 L/min, probe voltage of 4.5 kV, CDL voltage of - 35 V, Q-array voltages of 5, 5 and 20 V and Q-array RF of 150.

RESULTS: The following limits of detection and lower limits of quantification were obtained when 1 mL whole blood was extracted by SPE.

Opiate	LOD		LLOQ	
	Concentration (ng/mL)	S/N	Concentration (ng/mL)	S/N
Morphine	0.3	4	1	10
Codeine	0.3	5	1	15
6-Acetylmorphine	0.3	11	1	34
Dihydrocodeine	0.3	8	1	22

CONCLUSIONS: The method has now been used routinely in the laboratory for the analysis of blood samples from fatal and non-fatal cases.

Rapid Screen and Confirmation of Drugs and Toxic Compounds in Antemortem and Postmortem Specimens by LC Ion-Trap MS/MS - Opiates Example

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AIMS: Immunoassay is currently the method of choice for initial screen of drugs in biological specimens. Recent advances in the LC-MS/MS technology have provided an opportunity for the development of more specific approaches to achieve the ”screen” and ”confirmation” goals in a single analytical step. For this purpose, this study adapts the LC ion-trap MS-MS instrumentation for the screen and confirmation of 20 common opiates in postmortem specimens.

METHODS: Liquid-liquid and solid-phase extraction protocols were coupled to LC-ESI-MS/MS (Agilent LC/MSD trap XCT) using an Agilent Zorbax SB-Aq (2.1 mm x 150 mm, 3.5 µm particle) analytical column operated at 30°C. The analytes were eluted at a flow rate of 400 µL/min with a solvent mixture composed of methanol and water containing 0.1% formic acid. Positive-ion ESI MS^2 spectra and retention time for each of the 20 opiates were first established using 50 ng standards (Table 1). These spectra were then transferred to the library and

searched by the identification algorithm of the Agilent ion trap software for matching the spectra derived from unknown compounds found in the test specimen. Scores for Fit, Reverse Fit, Purity and Retention time are provided for each match. The limit of detection for each opiate was evaluated using samples spiked with decreasing concentrations.

Table 1. Detection of 20 opiates by LC/MSD Trap

Compound	Retention time (min)	Molecular wt. (m/z)	Precursor ion (m/z)	Product ion (m/z)	LOD (ng/mL) SPE	LOD (ng/mL) LIQ
Morphine-3ß-Glu	1.6	461.5	462.5	286.3	—	—
Normorphine	1.9	271.3	272.3	254.2	200	500
Morphine	2.2	285.3	286.4	201.2	50	50
Noroxymorphone	2.5	287.3	288.3	270.2	100	200
Oxymorphone	2.6	301.3	302.5	284.3	25	10
Hydromorphone	3.1	285.3	286.4	185.2	20	20
Nalorphine	4.9	311.4	312.4	270.2	25	10
Norcodeine	5.1	285.3	286.4	268.2	10	10
Dihydrocodeine	5.3	301.4	302.4	200.5	10	5
Naloxone	5.4	327.4	328.4	310.3	20	2.5
Codeine	5.7	299.4	300.5	215.1	10	10
Noroxycodone	7.0	301.3	302.3	284.2	20	50
Oxycodone	7.3	315.4	316.3	298.3	100	50
Naltrexone	8.1	341.4	342.6	324.4	5	10
Hydrocodone	8.6	299.4	300.5	199.0	50	10
6-Acetylmorphine	8.9	327.4	328.5	211.0	20	5
6-Acetylcodeine	12.8	341.4	342.4	225.1	20	5
Heroin	13.0	369.4	370.4	328.2	10	5
Norbuprenorphine	15.1	413.6	414.6	396.3	5	20
Buprenorphine	16.4	467.7	468.6	414.3	2.5	2.5

RESULTS AND CONCLUSIONS: This method provides a rapid, sensitive approach to isolate, screen and confirm a broad spectrum of opiates. The specificity of the method was evaluated using numerous antemortem and postmortem matrices. No significant interference was found at the retention time expected of the targeted compounds. This method significantly improves the efficiency of our routine laboratory operation that was based on a two-step (FPIA and GC-MS) approach in the past.

Validation of a Gas Chromatographic-Mass Spectrometric Method for the Simultaneous Determination of 13 Antidepressants and their Active Metabolites in Plasma and Application to Whole Blood

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AIMS: Monitoring of antidepressants provides cost-effective, rational use of psychiatric drugs. Both parent compounds and demethylated metabolites need to be determined as the latter also contribute to the overall therapeutic and toxic effect, and give information about time of ingestion, metabolic capacity, and compliance. The aim of this study is the validation of a GC-MS procedure for the simultaneous determination of relevant new antidepressants in plasma and evaluation of the applicability to forensic blood samples.

METHODS: Chromatographic and mass-selective conditions were optimized and selected ion mode was used for quantification of mirtazapine, viloxazine, venlafaxine, trazodone, citalopram, mianserin, reboxetine, fluoxetine, fluvoxamine, sertraline, maprotiline, melitracen, paroxetine, mcpp, norfluo–xetine, desmethylmianserin, desmethylmirtazapine, desmethylsertraline, desmethylcitalopram, and didesmethylcitalopram. Fluoxetine D6, mianserin D3 and paroxetine D6 were the internal standards. A HP 6890 GC-5973 mass-selective detector, a HP 7683 split/splitless auto-injector (at 300°C), and a 30 m x 0.25 mm i.d., 0.25 µm J&W-5ms column were used. The initial temperature was 90°C for 1 min, then ramped at 50°C/min to 180°C for 10 min and ramped again at 10°C/min to 300°C (5 min) at a constant helium flow (1.3 mL/min). The mass-selective detector temperatures were 300°C (transferline), 150°C (quadrupole), and 230°C (source). The ADs were extracted from 1 mL of plasma on a strong cation exchanger. After conditioning the sample (diluted with 4 mL of phosphate buffer pH 2.5) was loaded. After a wash with 4 mL of methanol compounds were eluted with 2 mL of 5% ammonia in methanol1. The validation procedure of Peters and Maurer2 was followed.

RESULTS: Most antidepressants as well as their heptafluorobutyryl-derivates were stable under different storage conditions. Calibrators (n=7) ranged from subtherapeutic to high therapeutic levels. Calibration curves were fit to a linear least-squares regression curve with a weighting factor of 1/x2. Reproducible recoveries both from plasma and whole blood (70 - 109%; 2 - 19 CV%) were obtained at three different concentration ranges (n=6). Intra-batch precision at LOQ (5 - 12.5 ng/mL depending on the compound), low, medium and high concentrations fulfilled the criterion of a relative standard deviation below 20% at LOQ and below 15% at the other levels. Inter-batch precision fulfilled this criterion except for sertraline at LOQ and for mcpp at low concentration. Accuracy ranged from 80 to 114%, except for mianserin and didesmethylcitalopram. No interferences were seen when analysing 15 blank samples from different individuals.

CONCLUSIONS: A GC-MS method for the simultaneous determination of 13 ADs and their active metabolites is validated. The method is also applicable to whole blood and thus to forensic samples with similar high and reproducible extraction efficiencies.

References:

1. Wille SMR, Maudens KE, Van Peteghem CH, Lambert WEE. J. Chromatogr. A 2005; 1098: 19-29; 2. Peters FT, Maurer HH. Accredit. & Qualit. Assur. 2002; 7: 441-449

Performance of a Validated Quantitative GC-MS Screening Method for Acidic and Neutral Drugs in Blood

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AIMS: Quantitative analysis of drugs and poisons in blood is a basis for interpreting the level of intoxication in human performance toxicology and postmortem toxicology. Instead of several narrow target analyses, broad scale drug screening with simultaneous quantification has been found to be a cost-effective, yet sufficiently accurate approach. We describe a validated method for acidic and neutral drugs based on full scan gas chromatography – mass spectrometry (GC-MS) for 1 mL whole blood samples.

METHODS: Post-mortem blood samples were extracted with ethyl acetate at neutral pH and trimethylsilyl derivates were generated with MSTFA + 1% TMCS. GC-MS was performed with 5973 inert MSD/6890 GC with performance electronics and Chemstation software (Agilent). Retention time locking was utilized, using the internal standard butalbital-D5 (6.4 min). Total runtime was 15.00 min on DB-5MS column (12 m x 0.20 mm, 0.33 μm). The measured spectra were purified using AMDIS (Automated Mass Spectral Deconvolution and Identification System) and subsequently searched against an in-house library containing over one hundred compounds. The spectra were also searched against an extensive commercial library (NIST05). The spectral match results from these two library searches and the respective blood concentrations were summarized in a single CAS number based table by Deconvolution Reporting Software (DRS, Agilent).

RESULTS: The method was validated for the quantification of more than forty drugs over a concentration range of 0.1 to 100 mg/L, applying three-point calibration. The limit of detection (LOD) was defined by using AMDIS; for most drugs LOD was 0.1 - 0.3 mg/L, being generally under therapeutic levels. Calibration was linear from subtherapeutic to toxic levels. Precision ranged from 4.2 to 17.6% and accuracy (bias%) from 2.1 to 15.3%. For the majority of drugs the extended uncertainty of measurement (2U) was below 30%, which is generally adequate for the purposes of postmortem investigation. The maintenance routine included daily tuning of GC-MS and weekly calibration of quantification for each analyte. In addition, a performance test sample was analyzed before each sequence and two in-house control samples, containing four analytes at two concentration levels, were analyzed once a week. The method has been in routine use with a daily loading of approximately 20-30 samples. Common findings include antiepileptics (e.g. carbamazepine and lamotrigine) and non-steroid anti-inflammatory drugs (NSAIDs), including oxicams and coxibs.

CONCLUSIONS: The described method has proved to be feasible for the routine quantification of commonly used acidic and neutral drugs, and it can also be used for searching large commercial libraries. The full scan method also provides an opportunity to expand on unknown compounds.

Determination of Opiates, Cocaine, Buprenorphine, Methadone, Propoxyphene and their Metabolites in Oral Fluid by LC-MS/MS

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AIMS: A rapid and sensitive method for the simultaneous analysis of Opiates, Cocaine, Buprenorphine, Methadone, Propoxyphene and metabolites in preserved oral fluid was developed and fully validated. Oral fluid was collected with the Intercept®, a Food and Drug Administration (FDA) cleared sampling device. The method comprised a solid phase extraction (SPE), followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Selectivity of the method was achieved by a combination of retention time, and two precursor-product ion transitions. Validation of the method was performed using 200 μL of oral fluid.

METHODS: The extraction utilized Waters Oasis® MCX 30 mg SPE cartridges. Samples were diluted with hydrochloric acid (0.8N) prior to loading onto the extraction columns. After washing with hydrochloric acid (0.1N), tetrahydrofuran and 50% methanol in water, the compounds were eluted with 10% ammonia in methanol. The samples were subsequently evaporated to dryness using a centrifugal evaporator under vacuum then reconstituted in 10 mM Ammonium formate containing 0.01% formic acid. Analysis of the extracted samples was performed on a LC-MS/MS system. The HPLC was a Waters® Alliance 2795 system equipped with a Waters XTerra® MS C18 (5 μm particle, 2.1 x 150 mm) column. Separation was achieved using a 10mM ammonium formate containing 0.01% formic acid and methanol solvent gradient at a flow of 300μL/min and a temperature of 40°C. The MS/MS system was a Waters® Quattro micro™ operated in ESI/MRM (Electrospray ionization/ Multiple Reaction Monitoring) mode analyzing a total of 43 ion transitions of 6-Acetylcodeine, 6-Acetylmorphine, Codeine, Dihydrocodeine, Heroin, Morphine, Anhydroecgonine Methyl Ester (AEME), Benzoylecgonine, Cocaethylene, Cocaine, Methadone, (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), Buprenorphine, Norbuprenorphine, Propoxyphene and deuterated internal standards.

RESULTS: Limits of quantification ranged from 0.1 to 1.0 ng/mL with intra mean accuracy ranging from 88.5% to 117%, inter-assay precision < 20% and inter-assay accuracy ranging from 91.1% to 114%. The method

was quantifiable over the range investigated up to 100 ng/mL for all analytes with intra mean accuracy for quality control samples spiked at three concentrations ranging from 92.5% to 113%, inter-assay precision and accuracy ranging from 0% to 9.4% and 94.4% to 107% respectively.

CONCLUSIONS: The method was subsequently applied to the analysis of Intercept® samples collected as part of on-going drug assessment programs and was successfully awarded ISO/IEC 17025:2005 accreditation.

Simultaneous Determination of Cocaine and Six Metabolites in Urine by Capillary Electrophoresis - Mass Spectrometry

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AIMS: Cocaine is the second most consumed drug of abuse in Brazil (after marijuana). In humans, cocaine is extensively metabolized, mainly by hydrolysis, n-demethylation and transesterification, which can result in psychoactive metabolites, such as norcocaine and cocaethylene (produced with the concomitant use of cocaine and ethanol). When traditional methods are used for the analysis of cocaine and metabolites many pitfalls may occur: anhydroecgonine methyl ester (a marker for crack cocaine use) could be produced in GC injector as an artifact; some metabolites with hydrophilic properties present poor retention in reversed phase liquid chromatography and low extraction recoveries from biological samples by liquid-liquid extraction or solid-phase extraction; some metabolites do not have chromophores that allow ultraviolet/fluorescence detection. The aim of this study was to develop a novel methodology, for determination of cocaine and six metabolites in human urine using capillary electrophoresis coupled to mass spectrometry (CE-MS).

METHODS: All the experiments were performed using fused-silica capillary with 80 cm x 50 mm i.d., temperature of 25ºC and applied voltage of 30 KV, electrolyte solution of 1 M formic acid. The ion trap mass spectrometer parameters were: spray voltage of 3 KV, sheath gas of 15 arb. units and liquid sheath containing 0.5% formic acid in (50:50) methanol:water delivered at a flow rate of 5 µL/min. Urine sample preparation was simply dilution with acetonitrile (0.5 mL of urine + 1.0 mL of acetonitrile), vortex shaking by 30 s and centrifugation at 300 g by 5 min. Samples were injected hydrodynamically 4 psi/10s.

RESULTS: The separation of cocaine and metabolites was achieved in less than 12 min. The LOD (signal-to-noise ratio = 3) were 100 ng/mL for cocaine and cocaethylene and 250 ng/mL for all other metabolites (benzoylecgonine, ecgonine, ecgonine methyl ester, anhydroecgonine, anhydroecgonine methyl ester). The LOQ (S/N = 10) was 250 ng/mL for cocaine and cocaethylene and 500 ng/mL for all other metabolites. The developed method presented good linearity for all analytes in the range from 500 ng/mL to 5000 ng/mL (coefficient of correlation greater than 0.98 for all compounds). The intra- and inter-day precisions of the proposed method were evaluated in three different concentrations (500, 1500 and 4000 ng/mL) and demonstrated relative standard deviations of < 20%, even at the LOQ.

CONCLUSIONS: The proposed method presented many advantages over established methodologies available in the literature allowing analysis of hydrophilic, thermally labile and chromophore-less compounds in the same analytical run. The procedure is simple, rapid, with inexpensive sample preparation and detection at or less than 250 ng/mL, which is suitable for confirmation of acute cocaine poisoning.

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Rapid and Sensitive Screening of Benzodiazepines in Serum Using Liquid Chromatography-APCI-Linear Ion Trap System

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AIMS: The benzodiazepines are primarily administered for their sedative-hypnotic effects. They are used as anxiolytics, anticonvulsants and are administered preoperatively for their anterograde amnesia effects (midazolam). However, hypotension, hypothermia, respiratory depression and death are associated with acute benzodiazepine toxicity. For the analysis of benzodiazepines in biological samples, a variety of spectroscopic, chromatographic and immunological methods are available. GC-MS is the method of choice, but unfortunately not for all. Some of them show thermal instability.Therefore, rapid and reliable screening of these substances is useful, especially for emergency cases.
We have developed rapid screening method for fast identification of seven most frequent benzodiazepines in East Czech territory as toxic agents (alprazolam, flunitrazepam, diazepam, midazolam, oxazepam, bromazepam, clonazepam).

METHODS: Serum samples were extracted by liquid-liquid extraction using commercial Toxi-Tubes B from

Toxi-Lab system or by SPE (mix phase MP1, Varian Corp). The HPLC (Rheos 2200 HPG high pressure gradient system, Flux Instruments AG) separation for this fast screening method was performed with 0.5% acetic acid/acetonitril gradient (60% CH_3COOH/ 40% CH_3CN to 80% CH_3COOH/ 20% CH_3CN during 5 minutes) on Discovery C18, 20 x 4.6 mm, 5 µm (Supelco). The total time of the analysis was 7 minutes. The mass spectrometer was operated in positive APCI mode. The corona discharge voltage was set at 2.5 kV. The capillary temperature was 275°C, sheath gas flow was 40 units, and auxiliary gas was set at 5 units. Full scan MS and MS^2 (30% normalized collision energy) spectra were acquired.

RESULTS: Using a setting of 30% collision energy was convenient for all identified benzodiazepines: precursor ion for alprazolam 309 $(M+H)^+$ ∀key product ions 281, 274, clonazepam 316∀270, 288; bromazepam 316∀ 288, 261, 236,181; diazepam 285∀ 257, 228, 222, 182; flunitrazepam 314∀ 286, 268; oxazepam 287∀ 269,259, midazolam 326∀ 291, 270, 244. The achieved limits of detection were satisfactory in a range of 0.05 (for midazolam in spiked serum samples) – 1 ng/mL (for the others) with S/N > 10. LL extraction recovery resulted in a range 60 - 85% for 0.5, 1, 5, 10 and 100 ng/mL spiked samples, SPE recovery was better than 85% for the same range of spiked serum samples.

CONCLUSIONS: We have good and reliable screening method for timely identification of problematic benzodiazepines in serum matrix. Additional detection methods including precursor ion and neutral loss scanning with high sensitivity in fast cycle time are the outstanding merits of linear ion trap system. In addition, high quality MS^2 and MS^3 spectra from real samples and good mass isotopic resolution in molecular peak in full MS mode are presented in this contribution.

A Benzodiazepine Screening Method Using a GC-MS Database

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AIMS: Benzodiazepines are one of the most abused pharmaceutical drugs. Since the benzodiazepines are rapidly metabolized, identifying the metabolites is also necessary for their detection. We have developed a database of 57 benzodiazepines including their metabolites to include mass spectra, mass chromatograms (m/z) and linear retention indices (LRIs)[1]. In this study, the applicability of the screening method using the database was investigated.

METHODS: The retention time (RT) usually differs depending on the column length even if the same or equivalent types are used. Although the RT is usually determined by analyzing a standard, standards for metabolites are often not readily available for most laboratories. Using a Shimadzu GCMS-QP2010 Plus, the RTs were estimated from measured RTs of n-alkanes and LRIs. The LRIs are constant for a particular column stationary phase and method parameters, which compensate for changes in column length and various instruments. To evaluate the accuracy of the RTs a mixture of clobazam, alprazolam, brotizolam, medazepam, lorazepam and midazolam samples diluted by ethyl acetate (5 ng/mL) was injected on the following columns: five [DB-5ms (30 m x 0.25 mm i.d., df = 0.25 um, Agilent Technologies, Inc., CA, U.S.A] and one [Rtx-5SilMS (30 m x 0.25 mm i.d., df = 0.25 um, Restek Corporation, PA, U.S.A]. An-alkane sample (C20 - C33) was used for the RT prediction reference.

RESULTS: The LRIs were obtained from one DB-5ms. They were 3027, 3164, 2284, 2458 and 2649, respectively, and the values were registered. The variations for other DB-5ms columns were from -3 to +7 and the average difference was 2.3. With the Rtx-5SilMS, the differences were within +2 to +10 and the average difference was 6.0. The time differences between calculated and measured were within -0.04 min to 0.06 min and the average was -0.02 min.

CONCLUSIONS: The evaluation results showed that the RT prediction was accurate enough for identification. In the screening analysis, the identification of benzodiazepines was possible using the mass spectra and predicted RT without the need for the analysis of standards.

Reference:

1. Nishioka et al., The Identification of Benzodiazepine Derivatives and their Metabolites in Urine and Blood Samples Using the GC/MS, The Pittsburgh Conference, Chicago, USA, 2004.

A Sensitive LC-MS/MS Method for the Determination of Hydrochlorothiazide and Chlorthalidone in Human Serum

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AIMS: A simple, sensitive and specific LC-MS2 method was developed and validated for the quantitation of hydrochlorothiazide (HCTZ) and chlorthalidone (CT) in human serum. The presented method is a useful tool for monitoring compliance in the therapy of hypertension and is currently being used to assay serum samples of patients.

METHODS: Samples (1 mL serum; 0.5 mL for dilution QC) were extracted by liquid-liquid extraction using a mixture of ethyl acetate and dichloromethane (80:20, v/v). Evaporated extracts were reconstituted in 200 µL of mobile phase consisting of 0.2% formic acid in water (pH 3) [A] and acetonitrile [B]. The chromatographic separation was performed on a reversed-phase Agilent Zorbax XDB-C8 column (75 x 4.6 mm i.d., particle size 3.5 µm) with gradient (60% A, 0.5 min; to 45% A in 9 min; to 60% in 0.2 min; total run time 11 min) at a flow rate of 0.2 mL/min. Detection was accomplished on a LTQ linear ion trap MS using electrospray ionization. The MS system was operated either in full-scan or in the MS2 mode. The parameters of MS were as follows: ESI probe with spray voltage 3.5 kV, ion transfer capillary heated to 275°C, normalized collision energy for MS2: 22% for HCTZ and the internal standard (IS) sulfadimethoxine, 21% for CT. The mass transitions m/z 296.1 → 205.0, m/z 337.1 → 319.0 and m/z 311.2 → 156.0 were used to measure HCTZ, CT and IS, respectively. Both diuretics were assayed in negative ion polarity mode, the IS in positive ion polarity mode.

RESULTS: Sulfadimethoxine was used as the IS because 1) it is not used for human treatment and 2) it is suitable as an IS for other diuretics (furosemide, spironolactone). The analytes were quantitated in the full scan MS^2 mode (precursor ion and neutral loss scanning). The assay exhibited a linear dynamic range of 0.5 – 200 ng/mL for HCTZ and 1.25 – 500 ng/mL for CT in human serum, respectively. The lowest calibrators were at the LOQ for the analytes. Quality control (QC) samples included low, med, high and dilution controls (x2) at 2, 80, and 200 ng/mL for HCTZ and 5, 200, and 500 ng/mL for CT. Six replicates of each were assayed for intra-assay precision (coefficient of variation (CV) ≤ 10%), and in triplicate on three different days for inter-day precision (CV ≤ 12%). The overall accuracy (relative error) was less than 10%. The selectivity of the method was studied by analysis of negative serum. No interfering peaks appeared in these chromatograms. Typical concentrations found in patient samples for HCTZ ranged from 79 – 216 ng/mL for HCTZ and 50 ng/mL for CT. In one case, HCTZ and furosemide were prescribed and also identified using full MS method.

CONCLUSIONS: An LC-MS/MS assay was developed and optimized for the determination of low concentrations of diuretics in human serum. The method can be used to quantify hydrochlorothiazide and chlorthalidone in human serum for studies in therapeutic drug monitoring (TDM).

An Expanded Opiate Panel for Urine Utilizing LC-MS/MS

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AIMS: Analysis of hydromorphone, morphine, oxymorphone, 6-MAM, oxycodone, codeine, and hydrocodone in urine utilizing LC-MS/MS has previously been presented [1]. This method was extended to include the synthetic opioid fentanyl. Fentanyl is often analyzed in a separate assay from the other opiates because the required detection limit for fentanyl is about an order of magnitude lower than the required detection limit for the other opiates. This disparity presents not only a dynamic range mismatch, but a potential challenge for a technique to meet the low levels necessary for detection and quantification. The objective of this project was to develop a single assay that would detect and quantify all opiates, including fentanyl, in urine.

METHODS: An Agilent 1100 LC stack was interfaced to a hybrid triple quadrupole/linear ion trap mass spectrometer. Separation was achieved on a 2 mm x 50 mm Aquasil C18 column and total run time was 6.4 minutes. Mobile phases A and B were water and ACN, respectively, with 0.1% formic acid added to each. Two MRM transitions, a quantifier and qualifier, were used for each analyte: hydromorphone, morphine, oxymorphone, 6-MAM, oxycodone, codeine, hydrocodone and fentanyl. Deuterated analogs of each were used as internal standards and spiked at 500 ng/mL. One MRM transition for each internal standard was monitored. Sample preparation consisted of a simple hydrolysis step and dilution. A 250 µL aliquot of urine was hydrolyzed and diluted to a final volume of 2 mL. Ten microliters were injected for analysis.

RESULTS: All eight opiates and their respective internal standards were successfully analyzed in a single LC-MS/MS method. The LOQ was about 0.25 ng/mL for fentanyl and 1 ng/mL for all other opiates. The linear dynamic range was from 5 ng/mL to at least 10000 ng/mL for all anlyates. Inter- and intra-assay precision were both measured. For inter-assay precision, five replicates each of the low and high QC standards were measured. For intra-assay precision, twenty measurements of the low and high QC standards were pulled from routine sample batches and the average and standard deviation calculated. The inter- and intra-assay precision values were better than 10%. To determine the accuracy of the LC-MS/MS method, forty samples were run using the LC-MS/MS method and the validated GC-MS method. The two sets of data showed good agreement, with a linear correlation of 0.90.

CONCLUSIONS: Transferring the opiate method from GC-MS to LC-MS/MS showed improvements in detection limits of at least 5x, as well as great time and cost savings from the simplified sample preparation

and reduction in number of samples that required re-analysis.

Reference:

1. Dahn, T., Shanks, K., and Sasaki, T. A.; SOFT Meeting, Austin, TX, 2006, Poster P27.

Determination of Opiates in Whole Blood by Liquid Chromatography – Ion Spray Tandem Mass Spectrometry (LC-MS/MS)

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AIMS: The aim of the study was to develop a rapid, sensitive and selective method for simultaneous determination of morphine, morphine-glucuronides, 6-monoacetylmorphine, codeine and other opiates and metabolites with LC-MS/MS.

METHODS: Whole blood spiked with deuterated internal standards, was mixed with ice-cold acetonitrile/methanol (85/15) followed by centrifugation. The opiates were separated on a XTerra® MS C18 column using gradient elution with a mobile phase consisting of acetonitrile and 5 mM ammonium formate buffer pH 3.1, at a flow rate 0.2 mL/min. The gradient started with the mobile phase of 3% acetonitrile subsequently increased to 60% acetonitrile. The running time was 10 minutes. The analytes were monitored on a triple quadrupole mass spectrometer (Waters) equipped with an ES (electro ion spray) source operated in the positive ionisation and multiple reaction monitoring (MRM) modes.

RESULTS: The results for limits of detection (LOD), limits of quantification (LOQ), accuracy and precision are presented in the table below. The accuracy and precision were evaluated at three concentration levels for the different compounds.

Substance	MRM (m/z)	LOD (mg/L)	LOQ (mg/L)	Day to day RSD (%)	Intra-day RSD (%)
Morphine	286.0 > 201.0	0,0003	0,001	3.8-4.6	1.8-4.1
Morphine-3-glucuronide	462.0 > 286.0	0,001	0,003	3.1-7.7	1.4-2.7
Morphine-6-glucuronide	462.0 > 286.0	0,001	0,002	1.5-8.1	1.5-1.9
6-Monoacetyl-morphine	328.0 > 211.0	0,0002	0,0003	3.0-6.5	1.0-5.2
Codeine	300.0 > 215.0	0,0002	0,0006	5.4-12.7	1.7-4.5

The results were compared with a GC-MS method using solid phase extraction, and showed satisfying accordance.

CONCLUSIONS: This method is timesaving because of the simple sample preparation. It is also suitable for analyses of opiates in decayed postmortem blood samples, where the GC-MS technique has been unsuitable. The method can easily be extended to include other opiates and metabolites (ethylmorphine, norethylmorphine, normorphine, norcodeine, codeine-glucuronide and ethylmorphine-glucuronide).

Simultaneous Screening and Quantitative Determination of Benzodiazepines (BZD) in Whole Blood by LC-MS/MS

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AIMS: The objective was to develop a sensitive and specific LC-MS/MS assay for screening and quantification in whole blood of benzodiazepines available in Denmark. 14 benzodiazepines and 2 metabolites were included in the method.

METHODS: Whole blood samples (0.5 g) were extracted with solid phase extraction (SPE) on Strata®-X-C columns from Phenomenex. The analysis was performed on a Waters 2695 Alliance liquid chromatograph coupled with a Micromass Quattro micro tandem mass spectrometer equipped with an ion-spray atmospheric pressure interface. LC-separation was performed on a C18-reverse phase column from Phenomenex (Synergi® 2µ Hydro-RP, 20x4.0 mm). Gradient elution was used, with the eluents consisting of a mixture of ammonium acetate pH 5.0, methanol, acetonitrile and formic acid. The MS/MS instrument was operated in positive ionization mode and data were recorded in the multiple-reaction monitoring (MRM) mode. Parent ions, the corresponding two daughter ions, retention time, cone voltage and collision energy for the 16 compounds were optimized and will be presented. Internal standardization was carried out using Diazepam-D5 as internal standard. Spiked blood samples (0.02 and 0.40 mg/Kg) of 16 compounds (7-amino-flunitrazepam, Nitrazepam, Flunitrazepam, Nordiazepam, Chlordiazepoxide, Clonazepam, Clobazam, Lormetazepam, Midazolam, Oxazepam, Alprazolam, Brotizolam, Bromazepam, Lorazepam, Triazolam, Diazepam) were used for the initial screening. Blood samples positive for benzodiazepines were re-analyzed and the compounds were quantified, using five spiked blood calibrators (0.010 – 1.00 mg/Kg).

RESULTS: The method was found to be selective for the 16 compounds in postmortem blood. No interfering peaks were observed in extracts of 3 different drug-free post-mortem blood samples. Interference with other compounds was minimized due to LC-MS/MS identification; retention time, two daughter ions per compound and ion ratios monitored between the daughters ions. LODs and LLOQs ranged from 0.001 to 0.008 mg/Kg and from 0.004 to 0.024 mg/Kg, respectively for the 16 compounds (0.5 g blood redissolved in 1000 µL ethanol, thus the LLOQ can easily be decreased). The calibration curves were linear in the measuring interval (0.01 – 1.0 mg/Kg). Linear regression correlation coefficients of the calibration curves were ≥ 0.993 for all compounds. Repeatability and intermediate precision [1] (expressed as RSD) for blood controls (0.02 and 0.40 mg/Kg) was in the range from 3 to 30%.

Precision was calculated using one-way analysis of variance (ANOVA) on duplicate measurements at each concentration level on six days. Accuracy was between 70 and 130% of target amount for a) certified reference controls (Medidrug Benzodiazepine S Level 1 and 2) containing 7-amino-flunitrazepam, Flunitrazepam, Nordiazepam, Oxazepam, Bromazepam, Lorazepam and Diazepam in the level from 0.01 to 0.6 mg/Kg and b) for spiked blood controls at 0.01, 0.5 and 1.0 mg/Kg. Extraction recovery varied from 78 – 130%. Ion suppression/enhancement was evaluated and was lower than 10% for all drugs. Ruggedness was tested on two in-house quality controls using a Youden test, and the method was found to be robust. The method is accreditated with the requirements of the ISO 17025 standard by an external independent organisation, DANAK.

CONCLUSIONS: A validated method has been described for analysing benzodiazepines available in Denmark. The method is found sensible, robust and reliable for screening and quantification of 14 benzodiazepines and 2 benzodiazepine metabolites in post mortem blood samples.

Reference:

1. F.T. Peters, O.H. Drummer and F. Musshoff, Validation of new methods. Forensic Sci. Int. Vol. 165 (2007) 216-224.

Quantitative Determination of Buprenorphine and Norbuprenorphine in Whole Blood by LC-MS/MS

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AIMS: To present a validated LC-MS/MS method for quantification of buprenorphine and norbuprenorphine in whole blood for routine use. Buprenorphine is used in the treatment of drug addicts in Denmark.

METHODS: 0.2 g blood was diluted with 0.1 M sodium carbonate buffer pH 9 containing 10% acetonitrile and transferred to an activated Certify Bond Elut extraction column. The extraction is performed automatically using Gilson Aspec XL4. Washing was performed with water, 0.1 M acetate buffer pH 4.0 and methanol before elution with dichloromethane : 2-propanol (80:20) containing 1% ammonia. D4-buprenorphine and D3-norbuprenorphine was used as internal standards. After evaporation to dryness the residue was dissolved in solvent. Spiked blood samples in the range 0.0002-0.0500 mg/Kg were used for the calibration curve. The analyses were performed on an Acquity UPLC-Quattro Premier XE Tandem MS/MS system (Waters). The separation column was a Waters Acquity 2.1 x 100 mm, 1.7 micron. The solvent consists of 2mM ammonium acetate pH 6.2 : methanol (2:8). The masses m/z 54.9 for buprenorphine (cone voltage (V):65, collision energy (eV): 45) and 82.9 for norbuprenorphine (cone voltage (V):55, collision energy (eV):43) were used for quantification and the masses m/z 83.5 and 100.9 were used as qualifier ions for detecting the ion ratio.

RESULTS: Quantification limit was 0.0002 mg/Kg. The calibrating curves were linear in the measuring interval, correlation coefficient > 0.99. The linearity was evaluated with polynomial regression. Recovery for spiked blood samples was 50-60 for buprenorphine and 80-90 for norbuprenorphine. The laboratory participates in an external quality control program.

CONCLUSIONS: A validated method has been described. The method is useful for quantification of buprenorphine and the metabolite norbuprenorphine. Results from autopsy cases as well as from living persons will be presented.

Development of a Method to Quantify Δ^9-tetrahydrocannabinol and its Metabolite 11-nor-Δ^9-carboxy-tetrahydrocannabinol in Whole Blood Using GC-EI-MS

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AIMS: With the widespread increasing use of cannabis a need arose within our laboratory to be able to quantify the active component Δ^9-tetrahydrocannabinol (THC) and its metabolite 11-nor-Δ^9-carboxy-tetrahydrocannabinol (THC-COOH) in whole blood. Method applications included the analysis of blood samples received in relation to sudden and suspicious deaths, road traffic offences, murders, assaults and drug assisted sexual assaults. For effective application within our laboratory a reproducible, robust and sensitive method was required.

METHODS: The developed method employs THC-d_3 and THC-COOH-d_3 internal standards prior to the addition of water and phosphate buffer. Liquid-liquid extraction with hexane:ethyl acetate (5:1) is followed by solid phase extraction (SPE) using Varian Bond Elut THC columns. Elution of separate THC/d_3 and THC-COOH/d_3 fractions is achieved using sequential gradient SPE eluents from 0.1% glacial acetic acid in hexane to 0.1% glacial acetic acid in hexane:ethyl acetate (6:4). The separate cannabinoid fractions are derivatized with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH) at 80°C affording stable derivatives. The samples are then evaporated under a slow flow of nitrogen to avoid loss of the volatile derivatized cannabinoids prior to heptane reconstitution. Analysis is carried out by gas chromatography electron impact mass spectrometry (GC-EI-MS) with a HP-5-MS fused silica column using selected ion monitoring to quantify THC and THC-COOH.

RESULTS: The method was employed to extract and

analyse both THC and THC-COOH calibration lines. Each line exhibited good linearity between 5ng/mL and 100ng/mL with correlation coefficients of not less than 0.995. The accuracy of the method was determined by extracting cannabinoid samples prepared by an independent scientist in our laboratory where the percentage errors were all less than 15%. The method was validated by quantifying cannabinoids within case blood samples where the determined concentrations were consistent with those reported by an independent laboratory.

CONCLUSIONS: The cannabinoid method has now been adopted within our laboratory for the routine quantification of cannabinoids in case blood samples.