PLENARY LECTURES

L01. Intoxications with plants and methodologies for the safety assessment of herbal medicines

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Since humans exist on Earth, there have been intoxications with plants. Toxic plants were even used to win battles and wars and famous historical figures and artists were victims of plants. It has been rumoured that Alexander the Great (356-323 B.C.) was intoxicated by white hellebore (*Veratrum album*, Liliaceae). The painter Vincent Van Gogh (1853-1890) drank frequently alcohol in excess and mainly absinthe prepared from *Artemisia absinthium* (Asteraceae) which caused some mental disorders. Since a couple of years, intoxications with medicinal plants, edible plants and herbal medicines are becoming more and more frequent. This is due to the fact that numerous persons are interested in all what is natural and believe that natural means safe! In Switzerland, for example, there are about eight times more intoxications with plants than with mushrooms. This is due to the poor botanical knowledge of many people who collect plants themselves. Confusions between *Allium ursinum* (Liliaceae) and *Colchicum autumnale*, and even *Convallaria majalis*, occur every year. Some people even can not distinguish *Taxus baccata* (Taxaceae) from Pinaceae species such as *Abies alba* or *Picea abies*. Herbal drugs which are on the market can also be victims of botanical confusions, as exemplified by the Chinese plant *Aristolochia fangchi* (Aristolochiaceae).

Microscopic analysis enables to detect very quickly falsifications of herbal medicines and some examples will be shown. TLC is also an important technique but the sensitivity is low. Hyphenated techniques such as LC/MS and LC/NMR are appropriate tools for the rapid identification of contaminants such as pyrrolizidine alkaloids which are hepatotoxic, anacardic acids which are strong allergens and many other classes of toxic plant constituants. Microcapillary NMR, due to its high sensitivity, will play a more important role in the future. The above mentioned technique can also be applied to the standardisation of herbal drugs.

L02. Genomic medicine: impact on drug efficacy and toxicity

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Interindividual variability in drug efficacy and toxicity is a major cause of therapeutic failure and contributes to high attrition rates during drug development. How can we learn to better predict the clinical efficacy and safety of an old or new drug? The interindividual variation of the human genome sequence can explain and predict the individual response in some drug therapies (pharmacogenomics, toxicogenomics), but environmental (*e.g.* smoking, other drugs) and host factors (*e.g.* age, sex, previous diseases) also contribute to variability for most drug treatments.

The search for genomic biomarkers for efficacy or adverse reactions has focused on variations of genes for drug metabolizing enzymes, in particular cytochromes P450 or MHC Class I genes for immune-mediated toxicities. A summary of pharmacogenomic biomarkers in the context of approved drug labels is available at http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm.

Examples of genetic biomarkers for efficacy are expression of HER2 and trastuzumab, variants of CYP2D6 and tamoxifen, wild-type K-ras and panitumumab and expression and mutations of EGFR and several EGFR-TKIs. Examples of genetic biomarkers for safety are HLA-allelic variants as well as in immune-mediated hypersensitivities and toxicities of abacavir (HLA-B*5701), and carbamazepine (HLA-B*1502). Examples for pharmacogenomic-based dose-prediction to enhance efficacy and improve safety are warfarin, tricyclic antidepressants, and efavirenz. These examples document the continuing development of pharmacogenomics/toxicogenomics as an important contributor to improved drug therapy.

Personalized medicine is a strategy to improve clinical outcome by precise diagnosis (*e.g.* subphenotypes of cancers) by optimizing drug choice and drug dose to the problem and need of the individual patient, by taking into account the individual's genome sequence and environmental and host factors. The reasonable hope is that this strategy will decrease the number of adverse drug reactions (ADRs) and increase the efficacy of drug therapy.

L03. Best practice *post mortem* forensic toxicology

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In recent years the science of forensic toxicology has evolved substantially due to an increasing understanding of its applications in death investigations and availability of increasingly sensitive analytical equipment. In parallel to this development there has been an increased understanding of the limitations in interpretations requiring a much more structured approach to analysing such data.

In most jurisdictions toxicology is requested on selected cases primarily to assist in the determination of the cause of death particularly when drugs (and other poisons) are suspected of being misused. However toxicology is also very useful when use of non-prescribed drugs may lead to behavioural changes precipitating aggressive or violent outcomes (*e.g.* benzodiazepines, amphetamines, cocaine etc.). Toxicology can also assist in establishing access to medications to detect underlying disease (*e.g.* epilepsy, heart disease or depression) and compliance to medication, effectively becoming therapeutic drug monitoring. In order to achieve the best result an appropriate choice of specimen is required and where possible an ante-mortem specimen taken in hospital to test a sample closer to the cause of the hospitalisation and to avoid the complications of *post mortem* redistribution. Interpretation of any toxicology data can only occur when the context of the case is fully known and medical records are available including access to medications and other substances. This is often lacking and needs to be included in any forensic toxicology case review.

Increasingly, speedy turn-around of cases can facilitate an investigation. In death investigations a quick result hours later or the next day can inform the medical investigator and the coroner as to the need, or otherwise, for an invasive autopsy, particularly if drugs are considered to have played a role (directly or indirectly) in the death. The increasing use of tandem MS techniques, particularly LC-MS/MS has allowed ultra-quick analytical systems to be developed for a large range of targeted substances. More recently this and related techniques now allow laboratories to include more substances at a lower concentration than that was hitherto possible.

The presentation will explore these developments, pros and cons of key specimens and provide delegates with information on how toxicologists can best assist the civil and criminal justice systems.

Keywords: *post mortem* toxicology, *ante mortem* specimens, redistribution, tandem-MS, medical history

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L04. Application of high resolution LC/MS for the analysis of pharmaceutical compounds, biological fluids and natural products

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Introduction: The identification of drug impurities, metabolites and degradation products forms a critical part of the drug discovery and development processes. More recently the analysis of biological fluids for changes in endogenous components concentrations, Metabonomics, has become an important part of the systems biology approach to mechanistic biology. The analysis of pharmaceutical products biological fluids and natural products requires high-resolution separation techniques and mass spectrometry to correctly profile, characterize and identify the components.

Aim: The purpose of our work was to demonstrate the application of high resolution chromatography employing sub 2 mm particles and elevated flow rates combined with high speed MS and accurate mass MS/MS for the analysis of pharmaceutical products and biological fluids.

Methods: Drug metabolites were obtained from development DMPK studies, urine and plasma samples were obtained from safety assessment studies and epidemiological studies. The samples were analysed using a Ultra Performance LC with 5 or 10 cm columns packed with 1.7 mm porous hydrid silica C18 material. The columns were eluted under gradient condition and eluent analysed by tandem quadrupole or QT of MS/MS.

Results: The data generated showed that by using the sub 2 mm material the chromatographic resolution could be improved by a factor of 3 and the sensitivity by a factor of up to 8. As the LC flow rate is significantly increased with these particles the analysis time could also be significantly reduced. In the data presented we will some the analysis of a natural product with a LC/MS chromatogram with a peak capacity of 1024 over 1 hr and urine metabonomics analysis using a 10-minute separation with a peak capacity of 700.

Conclusion: The use of sub 2 mm particle LC provides faster separations, higher resolution, improved spectral quality and increased sensitivity. These high resolution separations are ideal for the challenging applications of metabolite ID, metabonomics and natural products analysis.

Keywords: LC/MS/MS, QTof, metabonomics, metabolite ID, natural products

L05. Alcohol, drugs and driving: situation and update in Sweden

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Background: The punishable blood-alcohol concentration (BAC) for driving in Sweden is 0.20 ‰ (mg/g) and there is also a more serious offence (aggravated drunk driving) at 1.0 ‰ or more. For drugs other than alcohol, a zero-limit law was introduced in 1999, which meant that a person was charged and prosecuted if a controlled substance was unequivocally identified in a specimen of blood. An exception was made if the scheduled substance was a medicinal drug and was being used in accordance with a physician's ordination.

Aim: To give a broad overview of the development and present status of alcohol, drugs and driving in Sweden. The demographics of offenders and the spectrum of drugs used and abused by motorists as well as recidivism rates are reported. The occurrence of alcohol and/or drugs in blood samples from drivers killed in road-traffic crashes was also investigated.

Methods: Aliquots of peripheral venous blood were analyzed by well established methods; HS-GC (ethanol), GC-NP detector (pharmaceuticals) and GC-MS and LC-MS (illicit drugs). The results were entered into a forensic toxicology database (TOXBASE), which was the starting point for this epidemiological study.

Results: The number of blood samples sent by the police for toxicological analysis has now increased 14-fold after the zero-limit law came onto force. In 80-85% of cases the blood samples contain one or more banned substance either alone or together with ethanol. The offenders were predominantly men (85-95%) and their age depended on the drug identified in blood.

Drugs verified in blood samples	N	Mean age	Mean conc. mg/L	Median mg/L	Highest mg/L
Ethanol	32,814	40	1740	1780	5180
Amphetamine	9,162	37	0.77	0.60	22.3
Methamphetamine	644	36	0.34	0.20	3.7
Ecstasy (MDMA)	493	26	0.23	0.10	3.5
Tetrahydrocannabnol (THC)	7,750	33	1.9	1.0	36
γ-hydroxybutyrate (GHB)	548	26	89	82	340
Cocaine	160	29	0.069	0.05	0.31
Benzoylecognine	160	29	0.80	0.60	3.0
Morphine	2,029	33	0.046	0.03	1.13
6-acetyl morphine	52	33	0.016	0.01	0.10
Codeine	1,391	36	0.047	0.01	2.4
Diazepam	1,950	38	0.36	0.20	6.2

Illicit drugs (amphetamine and cannabis) and poly-drug use were common findings. Sedative hypnotics (benzodiazepines) and opiate analgesics were the most common prescription drugs identified in blood samples. In drivers killed in road traffic crashes 22% had a punishable BAC (> 0.2 ‰), 2.8% had used an illicit drug, 2% had used both an illicit and a licit drug and in 13.3% of cases one or more medicinal drug was identified.

Conclusion: People apprehended for drug-impaired driving in Sweden are often poly-drug users who combine alcohol or an illicit substance with a prescription drug, such as a benzodiazepine or opiate. During a 4-year period re-arrest rates were high (44%), especially in those drivers using amphetamine. Instead of traditional penalties for this traffic crime, much might be gained by psychological counselling, rehabilitation and treatment programs for substance-use disorder. Ignition interlock device can prevent an alcohol impaired driver from starting the vehicle and similar technology is urgently needed those impaired by drugs other than alcohol.

Keywords: alcohol, amphetamine, medicinal drugs, driving, crash statistics, fatalities

L06. Neurobiology of addiction

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Addiction can be defined as a disorder of motivated behavior. It is comprised of three stages: the acute drug effects (pleasure); the transition to addiction (repeated use); and the end-stage addiction (compulsion). Reinforcement models have tried to explain addiction based on negative or positive reinforcement theories which only illustrate part of the disease process. Chemically, the acute pleasurable effect is best linked to altered neurotransmission in key brain areas mediating reward. entral to drugs of addiction is their well recognized role in increasing the levels of dopamine in limbic and cortical systems. The presentation will explore different modality for drugs of abuse to result in this increased dopaminergic transmission, using stimulants, opiates, cannabinoids and nicotine as examples. More recently, other neurotransmitter systems and structures are gaining recognition in the processes of addiction: in particular glutamatergic pharmacology and brain peptides like CRF, NPY also participate in the complex behavioral changes associated with the switch from impulsive to compulsive use of the drug. In parallel, a progressive structural shift from limbic to amygdala and finally striatal activity is observed in end-stage addiction, reflecting a loss of control on drug intake. These changes underlie the pathological process that causes

the addict to switch from an occasional user to a compulsive user seeking the drug at all cost. The presentation will aim at giving both basic and advanced knowledge in the understanding of the neural and chemical substrates of addiction, focusing on the major classes of drugs of abuse.

L07. Past, present and future of anti-doping analyses

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Abstract not available.

L08. The athlete's biological passport

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Introduction: Modern olympism is born resting on the unwavering faith in a sound mind in a healthy body. Following this humanistic doctrine, doping is considered to be fundamentally contrary to the spirit of sport, with fair-play and health protection the two main goals of the anti-doping community. To fulfill these goals, anti-doping fight has quasi exclusively relied up to now on the paradigm to discover a doping substance in a biological sample of the athlete. Many see efficiency limits in this paradigm, mainly because novel drugs with a molecular structure very similar, if not identical, to endogenous substances are designed at an unprecedent pace today. As an alternative, indirect markers of doping reveal key modifications in physiological parameters induced by the doping product. Recent advances in the development and validation of indirect markers of doping, such as the introduction of multiparametric markers, the inclusion of heterogenous factors and/or potentially confounding effects, the use of the athlete's own previous measurements to define individual limits with the athlete becoming his own reference, the adoption of standardized protocols for sample collection and analysis, the setting up of quality control systems, the development and validation of probabilistic inference techniques to evaluate the value of the doping evidence, have all contributed to formalize this new paradigm into what is called today the athlete's biological passport (ABP). These last years, significant deviations from an individual baseline detected in an ABP has been used to target the athletes for direct anti-doping tests and, recently, to lead to an anti-doping infraction if no pathological condition can explain the irregularity.

Aim: To review the fundamental principles of the ABP and to define the conditions that a biomarker must fulfill to be part of the ABP.

Methods: A review of the literature on indirect evidence of doping, of passports obtained from various clinical trials as well as the discussion of some recent case studies.

Results: The development and validation of indirect biomarkers of doping is a fast growing field. Broadly, the ABP can be divided into three different modules: the athlete's haematological passport (AHP) based on indirect markers of blood doping, the athlete's endocrinological passport (AEP) based on a description of hormones secreted by the endocrine system and, as subsidiary of the AEP, the athlete's steroidological passport (ASP) composed of longitudinal steroid profiles. We see the introduction of a no-start rule based on the ABP – with the athlete temporarily excluded from competing – as a remarkable alternative to fulfill the two main goals of the anti-doping community - fair-play and health protection.

Conclusion: The advantages of adopting the ABP concept lie in the very fundaments of a doping free sport. Indeed, whereas a negative outcome of an anti-doping test does not prove that the athlete is clean, an athlete can use his passport to attest his fair-play via normal longitudinal profiles of biological parameters. Also, the same longitudinal profiles provide an invaluable tool to assist physicians in diagnosis of pathology. As such, the introduction of a no-start rule based on the ABP can ensure fair-play and health protection in elite sports. Finally, while the ABP has been primarily developed for anti-doping purposes, its application for clinical and medical care is straightforward in an evidence-based medicine paradigm that uses markers of disease.

Keywords: doping, indirect evidence, biomarkers

L09. Doping control analysis of peptides and proteins using LC-MS/MS

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Introduction: Peptides and proteins have become a central aspect of doping controls due to a considerable misuse of such therapeutics as outlined by recent findings of various erythropoietin (EPO) cases as well as confessions of athletes stating that human growth hormone (hGH), insulins, corticotrophins, and luteinizing hormone releasing hormones (LH-RH) have been subject of frequent misuse. Numerous peptide- or protein-based drugs are prohibited in sports according to the regulations of the World Anti-Doping Agency, and methods to detect these substances are urgently required. While some therapeutics are currently measured using immunological approaches, some target analytes allow their detection by means of sensitive and selective liquid chromatography-tandem mass spectrometry-based procedures, which adds another valuable tool to the doping control community.

Aim: The detection and characterization of peptide- and protein-based drugs using (high resolution/high accuracy) tandem mass spectrometry in doping control samples is aimed. In order to minimize sample consumption, labor, and costs, a comprehensive screening for these agents is preferred over itemized methods.

Methods: Various target peptides and proteins including LH-RH, insulins, and Synacthen were isolated from urine or plasma/serum using immunoaffinity purification strategies employing magnetic nano particles. The analytes were separated on a microbore or nano LC system using reversed-phase C-18 columns and detected using either a QTrap or LTQ-Orbitrap mass spectrometer operated in MS/MS modes. In case of more abundant protein drugs such as artificial oxygen carriers based on bovine hemoglobin, rapid and simple trypsin digestion followed by centrifugation and LC-MS/MS analysis was conducted.

Results: The combined and highly efficient isolation of various intact peptide and protein drugs as well as several metabolites allowed the detection of insulins, LH-RH, and Synacthen in one procedure. The sensitive nature of the assay enabled the detection of these compounds at detection limits ranging from 3-10 fmol/mL of urine, which proved sufficient to detect naturally occurring levels of insulin and its degradation products. LH-RH and Synacthen are normally not present in urine specimens, only after systemic administration, *e.g.*, intranasal or intramuscular. Hence, the qualitative evidence of their presence is considered sufficient for an adverse analytical finding. The proof of concept for all drugs was given by analyses of authentic administration study urine samples.

Conclusion: The use of LC-MS/MS to detect prohibited peptide and protein therapeutics in sports drug testing is a specific and selective complement to existing immunological methods. Although not all peptide hormones can (yet) be measured, an important subset of analytes is unambiguously identified.

L10. Cannabis testing in sport

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The inclusion of cannabinoids on the World Anti-doping Agencies Prohibited List in competition is a hotly debated issue. A short review of cannabinoid pharmacology and the neurobiology of the endogenous cannabinoid system will help to address the three criteria for inclusion of a drug on the prohibited

list. To be included, the substance must meet 2 of the following 3 criteria: enhance performance; pose a threat to the athlete's health; and/or violate the spirit of sport. Because the List only prohibits cannabinoids in competition, recency of cannabis use becomes an important issue. Differentiating new cannabis use from residual cannabinoid excretion is difficult in chronic, frequent users due to storage of Δ9-tetrahydrocannabinol (THC), the primary psychoactive component, in adipose tissue. The slow release of THC from the tissues is the rate-limiting step in the excretion of cannabinoids. In occasional cannabis users, the window of detection of ³15-ng/mL 11-nor-9-carboxy-THC in the urine is only 1 to 2 days. It has been proposed to quantify THC or its psychoactive metabolite 11-hydroxy-THC in urine after specialized hydrolysis procedures; however, this approach has been disproven as a means of identifying recent cannabis use. Perhaps analysis of alternative matrices such as oral fluid or blood could be employed to verify recent use, as windows of drug detection are short in these two matrices. A system might be designed to follow a positive urine cannabinoid test by an oral fluid or blood test, or two urine specimens might be collected over time to provide additional data. Bring your ideas to this lively discussion of cannabis testing in sport.

L11. Alternatives matrices (oral fluid, hair) in forensic toxicology and doping control: revolution with political issues or other specimens with financial issues?

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Introduction: Given the limitations of self-reports on drug use, testing for drugs is important for most situations, both for assessing the reality of the exposition and for evaluation of the level of drug impairment.

Methods: The presence of a drug analyte in a biological specimen can be used to document exposure or abuse. The standard in drug testing is the immunoassay screen, followed by the gas chromatographic-mass spectrometric confirmation conducted on a urine sample. In recent years, remarkable advances in sensitive analytical techniques have enabled the analysis of drugs in unconventional biological specimens such as oral fluid and hair. The advantages of these samples over traditional media, like urine and blood, are obvious: collection is non-invasive, relatively easy to perform, and it may be achieved under close supervision of medical staff to prevent adulteration or substitution. On-site tests (for oral fluid), ELISA and chromatographic methods coupled with mass spectrometry will be discussed.

Results: It appears that the value of alternative specimens analysis for the identification of drug users is steadily gaining recognition. The window of drug detection is dramatically extended to weeks, months or even years when testing hair. Hair analysis may be a useful adjunct to conventional drug testing in urine. Methods for evading urinalysis do not affect hair analysis. It has been claimed that the concentrations of many drugs in oral fluid correlate well with blood concentrations, which suggests that the oral fluid matrix can be a complementary matrix to blood since it represents active exposure.

Discussion: Numerous applications of alternative matrices have been developed in the recent years, including roadside testing of intoxicated drivers with oral fluid and documentation of forensic cases with hair, such as chronic poisoning, drug abuse and drug-facilitated crime. At the initial stages of the use of hair in workplace drug testing, external contamination was the key point. However, this was rather a financial fight between labs performing hair and those involved in urinalysis. Today, in doping control, oral fluid for cannabis testing could be of interest to document recent exposure (in comparison with long-term elimination in urine and the associated problems). As hair testing is recognized by most Courts of Justice, the lack of consideration by the world Anti-Doping Agency is to be classified as a political decision.

L12. Clinical chemistry and toxicology

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Diseases are either due to genes and/or the environment; the environment is divided in two: microbes & toxics. Many diseases including diseases related to toxics and drugs can damage progressively multiple organs without any patient warning or symptoms. Patients can loose roughly up to 40 percent of several organ functions without any degradation of their well being in normal resting condition. Therefore, clinical biomarkers are needed to evaluate the presence and the development of organ damage from any cause. Even more dramatically, in the USA, the third or fourth reported immediate cause of death is iatrogenic. Among them, half are due to drug interaction. Therefore, clinical chemist should use and/or develop efficient biomarker panels related to toxicants and drug monitoring programs to prevent such organ failure or even death. The "omic" paradigm and progression have brought new powerful tools especially in genomics, in proteomics and in metabolomics. Some have been extensively developed such as mass spectrometry. Latest mass spectrometry equipment, new analytical methods and software design offer the laboratory medicine tools to discover blood peptide modifications due to toxicants or screen for drugs and metabolites. Several recent developments shown during the presentation will highlight the impact of proteomics in clinical chemistry and toxicology.

L13. The necessity and interest of chemometrics

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Introduction: Chemometrics has been defined as the chemical discipline which uses methods of statistics, applied mathematics and informatics to:

- optimise the analytical chemical procedures;
- extract useful information from chemical data.

Chemometrics developed as a response to the increasing difficulty of many chemical problems – the number of factors influencing the system studied, the huge quantity of data that can be acquired on each sample, the complexity of the phenomena occurring in the samples, and also because of practical restrictions related to time and money.

Most instrumental techniques now used in Analytical Chemistry generate huge quantities of data. These techniques are sometimes "high-throughput" in that they can analyse samples at a fast rate (*e.g.* near-infrared), but much more often they are "large-bandwidth" in that they may be slow but produce enormous amounts of data for each sample (*e.g.* LC-MS, NMR, GCxGC-MS…).

Advantage can be taken of the fact that most types of instrumental variables are highly correlated and so contain common information. In the same way that it is some times preferable to use peak areas (sums of adjacent signal intensities) rather than peak heights (intensities of peak maxima), it is often even better to use an optimal combination of the original variables (weighted sums of all signal intensities).

Principal components analysis (PCA) gives one such optimal combination of variables.

PCA may be used as an exploratory data analysis method to detect relations among variables or among samples. It has also been adapted to serve as a means of generating regression models (principal components regression – PCR, partial least squares regression – PLS) or discriminant models (Factorial Discriminant Analysis – FDA, Partial Least Squares Discriminant Analysis – PLS-DA). The former models use the spectra to predict physico-chemical properties, while the latter use the spectra to class samples into different categories.

Both types of models are of obvious interest for authenticity controls, forensics, drug testing and drug fingerprinting.
Aim: Our aim in this talk is to present the basic idea behind these multivariate data analysis techniques and to give several examples of their applications.
Many difficulties are still encountered when using these methods.
1) One of the main problems is when dealing with megavariate (very wide) data sets which may go beyond available computer resources, *i.e.* storage and computation time.
As a solution to this problem, we will present the "principal components transform" paradigm, describe some of its properties and show examples of its application.
2) Until recently, chemometric datasets were usually collections of vectors (spectra, chromatograms). Many modern techniques (GC-MS, LC-MS, 3D-Fluorescence…) now produce a matrix or a cubic array for each sample. This has lead to the development of multi-way data analysis techniques, capable of extracting relevant information from such datasets. Applications of these methods will be presented.
3) The matrix calculus used in Chemometrics requires datasets in which each variable is in the same column in the data matrix for all samples. This leads to problems for the analysis of chromatographic data where peak positions change with temperature, pressure, flow-rate. Peak shifting methods that reduce the effect of the misalignment will be presented.
Conclusion: There are already many advantages in using Chemometrics to analyse chemical data. The co-evolution analytical instrumentation and data analysis techniques with the difficulty of the problems to be solved will make the use of Chemometrics not only advantageous, but necessary.
Keywords: chemometrics, multivariate data analysis

ORAL PRESENTATIONS

O01. Application of quantitative structure-activity relationships (QSARs) for modeling *post mortem* redistribution of structurally diverse drugs

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Introduction: *Post mortem* redistribution (PMR) constitutes a multifaceted process, which renders the analytical results of drug concentrations inaccurate to be interpreted by forensic toxicologists. Quantitative structure-activity relationship (QSAR) methodology is a useful tool that can serve in analyzing in a systematic way the information incorporated in the chemical structure of compounds in relation to their available biological data, supporting the reduction, refinement, and/or replacement of experimental studies.
Aim: The present study aimed to evaluate whether QSAR methodology could serve as an effective tool to estimate the ability of drugs to redistribute during *post mortem* period on the basis of their molecular, physicochemical and structural properties.
Methods: Multivariate data analysis (MVDA) was applied in data set of 78 structurally diverse drugs with available PMR data in the literature. Eighty-one physicochemical, molecular and structural specific constitutional properties were calculated by widely recognized computational packages.
Results: An adequate QSAR model (correlation coefficient/R2=0.65, cross-validated correlation coefficient/Q2=0.56, root mean square error of estimation/RMSEE=0.34) was established for 60 (77%) out of 78 drugs, which provided an informative illustration of the contributing molecular, physicochemical and structural properties in PMR process. Drugs with basic functionalities and enhanced molecular size, flexibily, lipophilicity and number of halogens were found to be susceptible to increased PMR.
Conclusion: Due to the high complexity of the PMR process, further QSAR studies need to focus on structurally related drugs to develop more specific models, which could serve as alternative tools to evaluate PMR for different chemical classes.
Keywords: *Post mortem* redistribution; QSAR; multivariate data analysis

O02. Drug interaction research on a *post mortem* database

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Introduction: Adverse drug interactions are a significant yet often predictable cause of hospitalization and morbidity. The simultaneous use of multiple drugs is especially common among the elderly and chronically ill. Polypharmacy is a challenge not only for clinicians but also for forensic pathologists and toxicologists carrying out *post mortem* investigation.
Aim: The purpose of the study was to determine the prevalence and nature of adverse drug combinations in the comprehensive Finnish *post mortem* toxicology database. The database was searched for drugs that are common *post mortem* findings and/or cause frequently poisonings. The role of adverse drug interactions was evaluated also for two particular drugs: venlafaxine was included because of its unexpectedly high fatal toxicity index in several studies, and warfarin because of the vast amount of interactions reported.
Methods: The drugs possessing potential to cause severe interactions were identified from the Swedish, Finnish, interaction X-referencing (SFINX) database. The case files in the *post mortem* database included a referral from a forensic pathologist, laboratory analysis results (drugs, drugs of abuse, alcohols etc.), and information extracted from the death certificate. The search results were based primarily on analysis of peripheral blood.
Results: An adverse drug combination was present in 0.71% of the 37,367 cases studied. In 23% of these cases, the concomitant use was concluded to have a possible role in the event of death. The most prominent combinations were those causing the serotonin syndrome, involving a β-blocker in combination with verapamil or diltiazem, and involving digoxin with diltiazem. The simultaneous use of warfarin and non-steroidal anti-inflammatory drugs was rare, but nevertheless 33% of the warfarin cases had at least one interacting drug present. In these cases, paracetamol was the most common finding, accounting for 49% of the cases. In venlafaxine cases, the presence of interacting drugs was also high (46%), and this percentage increased with the measured venlafaxine concentration in the blood.
Conclusion: A comprehensive *post mortem* toxicology database is a valuable source for extracting drug-interaction data. Both over-the-counter and prescription drugs are included, and the proof of concomitant use is based on laboratory analysis, not just on prescriptions. *Post mortem* epidemiology has proved to be a powerful approach to reveal adverse drug interactions and to improve drug safety in general.
Keywords: adverse drug interaction, *post mortem* database, venlafaxine, warfarin

O03. Investigation of chronic cocaine consumption from *post mortem* hair analysis

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Introduction: Chronic use of cocaine is known to cause physical (*e.g.* cardiac and lung damage) and psychological or behavioural (*e.g.* depression and psychosis) problems in individuals. Cocaine use has become increasingly prevalent in society and the effects of chronic use of the drug have been recognised in post mortem cases.
Aim: Results from multi-analyte hair analysis of 399 *post mortem* (PM) cases from 2001–2008 were examined. The types of trauma cases submitted

for analysis included sudden adult death, hanging, drowning, train and traffic collisions, stab or gunshot wounds, fall from height, cardiac event and excited delirium. Previous use of cocaine, opioids, amphetamines, diazepam and frequently prescribed medications was determined by hair analysis. For this presentation the cocaine-positive results were studied to determine the patterns of cocaine use by individuals. A general guideline of cocaine concentrations in hair was developed and used to distinguish low-level or recreational use ("low" range) from excessive cocaine use ("upper" range) for this population. The cases where cocaine and/or metabolites were detected in conventional PM samples such as blood and urine (bio-fluids), where cocaine or "crack" use was stated in the case history and where cocaine was detected in the hair were compared.

Method: Hair from *post mortem* cases was segmented and analysed using a validated method for multi-drug analysis, described previously. The drugs detected were grouped into categories (amphetamines, opioids and opiates, cocainics, diazepam and frequently prescribed drugs). The concentration range guideline was determined by using the cocaine concentration results obtained; the minimum to 25th percentile was set as the "low" range and 75th percentile to maximum was set as the "upper" range.

Results: Cocaine was detected or indicated in 60% of the cases submitted and was the prevalent drug detected in hair samples. From 239 cocaine-positive cases, cocaine was detected in the hair of 95% of the cases, but use was stated in only 19% of case histories and detected in 17% of bio-fluid samples. Cocaine use was determined by the presence of cocaine and benzoylecgonine (BE) in 84% of hair cases and the results where BE was absent were reported as cocaine exposure. The cocaine concentrations detected ranged from 0.2 – 649 ng/mg of hair, and the BE concentration ranged from 0.2 – 276 ng/mg of hair. Cocaine/crack use was common in both poly-drug users (115 from 208 cases) and single-drug users (47 from 96 cases). The frequency of any drugs detected in the bio-fluids was higher (85% of cases) in the "upper" cocaine range cases (19.4 - 385 ng/mg) in comparison to the "low" user cocaine range cases (0.2 – 0.6 ng/mg) which comprised of 46% of positive bio-fluid cases. Cocaine used alone was detected in 39% of "low" range hair cases and 18% of "upper" range cases for this population.

Conclusions: Chronic cocaine use was indicated by hair analysis in approximately 50% of trauma cases submitted and effects such as depression, excited delirium and cardiac problems were evident from the results. The analysis of bio-fluids and case information underestimated the rate of cocaine use in comparison with hair analysis. Hair analysis is an important diagnostic tool in identifying chronic cocaine and is useful in explaining the cause of death.

Keywords: *post mortem* hair analysis, chronic cocaine use, diagnostic use

O04. Methadone concentration in liver and *post mortem* blood

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Introduction: Methadone is a common cause of drug related fatalities particularly if tolerance to its toxic effects has not developed with regular use. Methadone is subject to *post mortem* redistribution, consequently, the concentration of methadone measured in *post mortem* blood may be higher than was present in the blood at the time of death.

Aim: To investigate the relative concentration of methadone in liver and blood to provide a better understanding of the inconsistent and significant effects of *post mortem* changes.

Method: The liver was digested with tris buffer, stirring for one hour at about 60 ºC. The methadone was extracted from 0.2 mL of blood and liver digest using 0.1 M phosphate buffer (pH6) and Bond Elut Certify SPE columns (3 mL) coupled with the Zymark Rapid Trace SPE Workstation. Quantitation was undertaken by GC/MS/NPD. The details of the deaths were obtained from the Coronial Service files.

Results: The level of methadone was determined in the liver and blood of 215 cases between 1995 and 2008. Coroners' findings determined that 182 cases were drug related deaths, of which 34% had been prescribed methadone. The methadone had been prescribed for less than 15 days in nine cases. There was evidence of suicide in nine cases. Evidence of IV abuse was found in 45% of cases. Methadone was the only central nervous system depressant found in 24% of cases who had been prescribed methadone and in 30% of the cases that had used diverted methadone. The prescribed dose of methadone (38 cases) was 30 to 470 milligrams per day (median: 90). Delay between the time the body was found and autopsy (154 cases) ranged from 2 to 78 hours (median: 20 hours). Males accounted for 71% of those who had used diverted methadone and 51% of those prescribed methadone. The concentration of methadone in the peripheral venous blood and in the liver, expressed as the liver to blood concentration ratio (L/B), follow. Drug related fatalities: Diverted methadone (98 cases) blood ranged 0.01 to 1.4 mg/L (median: 0.39 mg/L), L/B ranged from 2 to 200 (median: 6). Prescribed methadone (51 cases) blood ranged from 0.16 to 6.4 mg/L (median: 1.0 mg/L), L/B ranged from 1 to 35 (median: 6). Death not related to drugs: Diverted or prescribed (23 cases) blood ranged from 0.09 to 1.5 mg/L (median: 0.90 mg/L), L/B ranged from 3 to 20 (median: 9).

Conclusions: The concentration of methadone in liver was found to be, on average, about six times higher than that found in peripheral blood. However, the range of results was significant (1 to 200 times higher). The findings of this study serve to highlight the difficulty in interpreting *post mortem* blood methadone concentrations.

O05. Problems of interpretation of *post mortem* toxicological findings in babies and young children: a report of two cases

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Introduction: In forensic cases concerning adults, the toxicologist can rely on a multitude of data documenting therapeutic, toxic as well as lethal blood concentration ranges for most pharmaceuticals. In contrast, for children such common reference values for pharmaceutical plasma concentrations are not established. The dosage of children's medication is most of the time just estimated considering the patient's age and body-weight as well as previous clinical experiences. Furthermore, only few data concerning the transfer of pharmaceuticals from mother to child via the placenta and/or breastfeeding are available. Therefore the interpretation of plasma-concentrations in children, especially in (suspected) fatalities, can be difficult. In the first case, a few days old baby was found dead in its mother's car. In the apartment of the mother a lot of pharmaceuticals were found. In the second case, a 12 month old child, which had had diarrhoea two days before, suddenly showed severe nose-bleeding with foamy blood. Shortly after, the child died.

Aim: Based on these cases, the purpose of our work was to first emphasize the complexity of interpretation of determined pharmaceutical concentration in blood samples of children. Furthermore, a simple and easy method for the identification and quantification of the antidepressant mirtazapine in plasma and several organs is presented.

Methods: For the quantification of mirtazapine, 2 ml phosphate buffer (pH 9) was added to 1 ml blood or 1 g organ. For extraction, a mixture of 1-chlorbutane and iso-propanol (9:1, v/v) was used. After evaporation, the residue (solved in ethyl acetate) was analysed by GC/MS.

Results: In the first case, the pharmaceuticals tramadol (analgetic), metamizole (analgetic), ibuprofen (analgetic) and thiopental (barbiturate) were detected in urine. By chemical analysis of femoral blood as well as different organs, metamizole was found in concentrations of generally > 1 mg/l, while thiopental was detected in a concentration of 0.06 mg/l. In the literature, metamizole-intoxications in adults were only observed at concentrations exceeding 20 mg/l. The detected thiopental-concentration was very low (therapeutic: 1-5 mg/l), and could be traced back to a documented

administration to the mother preceding her caesarean section in hospital. Histological examination showed no pathological findings. In summary, the detected pharmaceuticals alone or even in combination cannot be considered fatal. An oral administration by the mother could not be proven. In the second case, mirtazapine and diclofenac (analgetic) were detected in blood in concentrations of 1.6 mg/l and 2.0 mg/l, respectively. After being confronted with these results, the mother confessed to an oral administration of 3 tablets (90 mg) to the child. The toxicological interpretation was difficult. Because therapeutic concentrations for mirtazapine in adults range up to 0.3 mg/l, intoxications are only observed at concentrations higher than 1 mg/l. However, in literature no mirtazapine-mono-intoxications have been described. Even the ingestion of a 50-fold of a normal daily dose was survived. For children no corresponding data exist. To solve the case, we performed special histological examinations, which provided evidence for a cardiac disease as cause of death.

Conclusion: The interpretation of determined pharmaceutical concentrations in blood of children is very complicated because of the rare data concerning therapeutic/toxic ranges, the transfer of substances from mother to child via the placenta and/or by the way of breastfeeding.

Keywords: mirtazapine, intoxication, children, therapeutic, pharmaceuticals

O06. Fast targeted screening of drugs and poisons in *post mortem* blood

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Aims: Targeted screening methods that cover a broad range of common drugs and drugs of abuse are essential for forensic laboratories. The most common techniques used are immunoassay techniques, GC-MS, and more recently LC-MS(MS). The major advantages of immunoassay techniques are their easy use and fast turnaround time, however, mass spectrometric techniques are more selective and/or sensitive, and technically more demanding. The aim of this study was to develop a LC-MS/MS based screening technique that covers the most common drugs observed in forensic analysis, using fast extraction method combined with fully automated data processing.

Methods: After liquid-liquid extraction (LLE) of 100 µL of post mortem blood, 105 of the most common drugs and drugs of abuse were separated using a Shimadzu Prominence HPLC system with an C18 separation column (Eclipse XBD C18, 4.6 × 150 mm, 5 µm), using gradient elution with a mobile phase of 50 mM ammonium formate buffer pH 3.5 / acetonitrile. The drugs were detected using an Applied Biosystems 3200 Q-TRAP LC-MS-MS system (ESI, MRM mode). The method was fully validated according to international guidelines. Quantification data obtained using calibration curves were compared to a one point calibration. Data processing was performed using a custom designed macro based on Analyst Reporter 2.0 software.

Results: The assay was found to be selective for the compounds of interest. The calibration range spanned from therapeutic to potentially toxic concentrations described for these drugs. With a few exceptions, recoveries were typically > 70%. Accuracy, repeatability and intermediate precision were within the required limits for most analytes. Using a one point calibration, most analytes obtained similar accuracy and precision compared to a full calibration. Data processing was automated based on custom built settings. All quantifier transition peaks matching the retention times within 2% maximum difference that resulted in a calculated concentration above the lowest calibrator were reported as tentatively positive for further checking. In the scenario where the qualifier ion matched the MRM ratio obtained from in batch quality control samples within the EU mass spectrometry guidelines, the drug was highlighted in green. In the scenario where the MRM ratio did not match, the drug was still shown as tentatively positive, but highlighted in yellow. Peak review including MRM ratio was assessed for all tentatively positive peaks.

Conclusions: The presented LC-MS-MS assay has proven to be applicable for determination of the studied analytes in blood. The fast and reliable extraction method combined with automated processing gives the opportunity for overnight turnaround times.

Keywords: LC-MS-MS; Screening; *post mortem* blood

O07. Strychnine: old poison, new deaths

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Introduction: Strychnine poisoning is relatively rare in Australia. Strychnine is a potent central nervous system stimulant and convulsant and is primarily used for extermination of rodents. Death has been reported to occur following ingestion of 60-100 mg by an adult. Death occurs by paralysis of the muscles involved in respiration (breathing), however, even with greater amounts of strychnine ingestion, survival is possible with appropriate treatment (Moffat *et al.*, 2004). This data summarises all deaths as a result of strychnine poisoning at the Victorian Institute of Forensic Medicine over an 18 year period.

Methods: Most cases were subject to a full autopsy and all cases had a thorough toxicological examination. 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 including strychnine were quantified using either HPLC/DAD or GC/MS. Further tests for alcohol and other volatiles were separately conducted. Strychnine was determined in *post mortem* blood in all cases. Concentrations were also measured in stomach contents and liver in a number of cases.

Results: From some 50,000 *post mortem* examinations conducted over an 18 year period, only 15 cases involved the detection of strychnine. All cases were males and except for one case the cause of death was given as toxicity to strychnine in all cases. Case 15 involves the potential administration of strychnine as intentional (*i.e.* possible homicide). The following table shows the age, gender, manner of death as well as the *post mortem* blood, liver concentrations and stomach content of strychnine.

Case	Year	Age	Manner of death	Strychnine [blood] mg/L	Strychnine [liver] mg/kg	Strychnine [stomach contents] mg
1	1990	74	Suicide	12	NA*	175
2	1992	78	Suicide	0.6	0.2	2000
3	1992	50	Suicide	1.5	NA*	170
4	1993	30	Suicide	0.8	NA*	249
5	1994	74	Suicide	1.0	9	4
6	1994	87	Suicide	2.0	NA*	600
7	1994	68	Suicide	1.0	NA*	30
8	1996	80	Suicide	2.6 (cavity blood)	NA*	Trace Detected < 1 mg
9	1998	36	Suicide	1.1	NA*	Detected
10	2000	24	Suicide	3.8	NA*	NA*
11	2003	79	Suicide	1.2	NA*	NA*
12	2007	71	Suicide	3.9	NA*	NA*
13	2008	19	Accident	1.0	4.3	NA*
14	2008	21	Accident	1.5	3.0	NA*
15	2008	46	Unknown	1.8	12	362

* NA – Not available for testing or not performed, Femoral blood used unless indicated otherwise.

The concentration of strychnine in the blood ranged between 0.6 mg/L and 12 mg/L. The literature states that concentrations less than 2 mg/L may be toxic and levels above 10 mg/L may be lethal. Only 28% of the cases in

Victoria in the last 14 years were greater than 2 and only one case was above 10 mg/L, a level which is usually considered fatal. Strychnine was the only drug at toxic concentrations found in all cases.

Conclusions: The concentrations of strychnine in these cases showed all but three cases to be less than 2 mg/L, and only one greater than 12 mg/L. This suggests that concentrations in femoral blood may be toxic at lower levels than previously described .

Keywords: strychnine, death, *post mortem* blood

O08. Metals distribution in tissues of deceased individuals

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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. Hair and nail are of interest as a cumulative biomarker of long term exposure but they may be affected by exogenous contamination. Moreover, in some forensic cases, when blood and urine are not available it would be extremely useful to document metal distribution in deep tissues.

Methods: To obtain the usual values of 34 elements in the normal human body, the amounts of 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, La, Gd, W, Pt, Hg, Tl, Pb, Bi, U, were determined in 20 female and male human cadavers, whose ages ranged from 19 to 57 years. Inductively coupled plasma-mass spectrometry (ICP-MS) was used for frozen autopsied human organs samples (cerebrum, heart, kidney, liver, lung, and muscle). Five hundred milligrams of tissues were digested at 70 °C with pure nitric acid and diluted (nitric acid, butanol, and triton) before analysis. Indium and Rhodium were used as internal standards. Bovine liver certified material was employed to ensure optimal quality of the analysis. As an application of this multi-elementary tissue determination, the results obtained from a liver biopsy are presented in a case of gadolinium nephrogenic systemic fibrosis (Gd-NSF).

Results: Linearity was excellent within a wide range with a slope higher than 0.99 for all the elements. Detection limits ranged from 8 pg/g for uranium to 28 ng/g for boron. The intra-assay and the inter-assay inaccuracies, measured as the variation coefficient were below 5 and 10% respectively. All elements showed log-normal distribution. Distribution of numerous metals in the human body was almost as uniform as B, V, Ni. Very large quantities of Cd were found in the kidney. Metabolic organs contained the highest level of some elements as Mn, Co, Mo, Pb. Concentration of Al was greatest in the lung, tissue exposed to the exterior. These results are discussed and compared to the limited literature data. As an application, in the first French Gd-NSF case we reported, the liver Gd content was 200 times higher than the normal concentration. For forensic toxicology, metal and metalloid tissue analyses are also of major interest in many circumstances to document death (body fluids not available, embalmed body).

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

O09. Platinum in internal organs and blood after cisplatin overdose

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Introduction: Cisplatin is widely used as a chemotherapeutic drug for the treatment of numerous different types of cancers, as it possesses strong antitumor activity. Unfortunately, it is also very toxic and has severe side effects: emesis, renal failure, myelosuppression, deafness etc. Cisplatin overdosing may lead to serious health complications and even death; however, cases of cisplatin overdose are rather rarely reported in the literature. This paper presents a case of a young woman who was given an overdose by mistake: she received a total of 400 mg/m2 cisplatin over the course of four days (*i.e.* 100 mg/m^2/day) – which was four times what she should have received. The mistake was noticed just at the end of chemotherapy. In spite of treatment administered, including plasmapheresis, the patient died on the 19th day after chemotherapy due to sepsis.

Aim: The aim of this work was to determine platinum (Pt) in the blood and internal organs in the above mentioned cisplatin overdose case, using inductively coupled plasma optical emission spectrometry (ICP-OES).

Methods: Blood and internal organs: liver, kidney and stomach were collected during autopsy. Prior to Pt determination, samples of investigated material were microwave digested. ICP-OES determination was carried out at emission lines 214.423 and 265.945 nm. The detection and quantification limits (LOD and LOQ), relative standard deviation (RSD) and recovery of Pt (added at the level of 2 µg/g of blood, liver and kidney) of the analytical method was calculated.

Results: The concentration of Pt in blood, liver, kidney and stomach of the dead patient was 0.3, 5.5, 6.6 and 2.9 µg/g, respectively. The LOQ at lines 214.423 and 265.945 nm was 0.11 and 0.22 µg/g of blood and internal organs. The recovery for both analytical lines and all studied materials was in the range of 95.6 – 102.9%, and the RSD in the range 4.4 – 9.5%.

Conclusion: The highest Pt concentration was found in the kidney, then in the liver; the concentration in blood was much lower. Pt concentrations in internal organs in cases of cisplatin overdose may be determined by the ICP-OES method with sufficient precision and accuracy.

Keywords: cisplatin, overdose, internal organs, ICP-OES

O10. Post mortem (PM) blood & vitreous ethyl glucuronide (EtG) & ethyl sulphate (EtS) quantitation using LC-MS/MS

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Introduction: There is evidence that bacterial contamination may effect EtG & EtS blood concentrations. There is a low risk of this occurring in vitreous.

Aims: To investigate vitreous as an alternative matrix for the quantitation of EtG & EtS.

Methods: a) Clinical sample groups - Analysis was performed on samples in which further investigation of potential use of alcohol use was required. High alcohol consumption: n=9, with PM blood alcohol > 200 mg/100 ml & no evidence for putrefaction. Low-level alcohol consumption: n=10, with a blood alcohol concentration of < 100 mg/100 ml, & vitreous alcohol < 100 mg/100 ml with no evidence for putrefaction. Low blood alcohol levels & evidence of putrefaction: n=7, blood alcohol of < 100 mg/100 ml, no alcohol detected in vitreous, evidence of putrefaction with no history of alcohol consumption. No alcohol consumption suspected: n=5, with no suspicion of alcohol consumption. **b) Analytical Methods**: Blood-1.2 ml methanol, 40 µl pentadeuterated-EtG (EtG-D5) & 130 µl pentadeuterated-EtS (EtS-D5) were added to 0.6 ml whole blood. The samples were vortexed for 30 seconds, frozen for 20 minutes (– 20 °C), centrifuged at 1159 G for 5 minutes & supernatant dried down (31 °C). The residue was re-suspended in 1 ml of 1% formic acid in H2O & vortexed for 15 seconds. The samples then underwent SPE clean up using 200 mg Clean Screen extraction columns. The supernatant was dried down at 31 °C & reconstituted in 150 µl of 0.1% formic acid in H2O. **Vitreous**–100 µl 0.1% formic acid in acetonitrile, 15 µl EtG-D5 & 15 µl EtS-D5 was added to 100 µl of vitreous. The samples were vortexed for 30 seconds & centrifuged at 1159 G for 5 minutes. **LC-MS/MS**-Samples were run on the ABI 3200 QTRAP using a Phenomenex Gemini 105 mm × 2 mm × 5 µm C18 column with mobile phase 95:5 (0.1% formic acid in H2O: 0.1% formic acid in acetonitrile). The EtG parent ion (221.2) with 2 qualifier ions (85 & 74.9) & EtS parent ion (125) with 2 qualifier

ions (97 & 80) were used for quantitation. The EtG-D5 parent ion (226.2), qualifier (85.1) & EtS-D5 parent ion (130), qualifier (98) were used as internal standards. In whole blood the retention time for EtG & EtG-D5 was 1.70 minutes while EtS & EtS-D5 was 2.12. In vitreous the retention time for EtG & EtG-D5 was 1.20 minutes while EtS & EtS-D5 was 1.24.

Results: The mean blood alcohol concentration in the *high alcohol* consumption group was 292 mg/100 ml & vitreous 337 mg/100 ml. The mean blood EtG concentration was 2.97 mg/L, & vitreous 2.57 mg/L. The mean blood EtS concentration was 1.6 mg/L, & vitreous 1.48 mg/L. There was no statistical difference in alcohol concentrations between the 2 low concentration alcohol groups (mean 43 mg/100 ml *vs.* 12 mg/100 ml). In the Low-level alcohol consumption group the mean blood EtG concentration was 2.1 mg/L, & vitreous 3.1 mg/L. The mean blood EtS concentration was 1.6 mg/L, & vitreous 1.9 mg/L. In the Low blood level alcohol + evidence of putrefaction group EtG & EtS were not present in any of the cases (EtG: Blood LOD=0.08 mg/L, vitreous LOD=0.09 mg/L; EtS: blood LOD=0.03 mg/L, vitreous LOD=0.07 mg/L). Neither EtG nor EtS was present in the *no alcohol* consumption suspected group.

Conclusion: This is the first report describing the analysis of EtG & EtS in vitreous PM samples using LC-MS/MS. As with blood there is good discrimination in the problematical area for interpretation of PM blood alcohols <100 mg/100 ml. The research shows vitreous maybe a viable alternative matrix to blood for the analysis of EtG & EtS, thus avoiding the potential problems seen with bacterial contamination in blood.

Keywords: EtG, EtS, Vitreous, Blood and LC- MS/MS.

O11. Potassium chloride and gamma-hydroxybutyrate distribution: about an unusual case

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Case report: Madam X, 35, anesthesiologist, treated for severe depressive disorder with fluoxetine, was found dead in her office, several vials of potassium chloride empty at her side. Despite resuscitation attempts and several injections of gamma-hydroxybutyrate to release intra-cellular potassium, death was reported two hours later. A blood sample was taken during the examination of the scene of death, supplemented by subsequent samples at the autopsy.

Methods: Potassium was measured in blood and vitreous humor by ICP/MS. After precipitation with acetonitrile and derivatization with BSTFA, the gamma-hydroxybutyrate was measured by GC/MS. Finally, blood was screened for drugs of abuse and pharmaceuticals by routine procedures including immunoassays, liquid chromatography coupled with a diode array detector (HPLC-DAD) and gas chromatography coupled with mass spectrometry (GC-MS).

Results: are presented in table 1:

	Potassium	Gamma-hydroxybutyrate	Fluoxetine
Subclavian blood (scene of death)	64.8 mEq/L	-	
Femoral blood (autopsy)	91.8 mEq/L	533 mg/L	1.47 mg/L
Vitreous humor	13.8 mEq/L	491 mg/L	

Conclusion: These results show a massive exogenous exposure to potassium chloride as well as gamma-hydroxybutyrate absorption, confirming data from the anamnesis. The blood concentration of fluoxetine is also supra-therapeutic. The authors discuss changes in concentrations between different blood compartments and vitreous humor in relation to the phenomena of *post mortem* redistribution.

Keywords: suicide / potassium chloride / gamma-hydroxybutyrate / *post mortem* redistribution

O12. Determination of ethylene glycol, glycolic acid and formic acid in blood and urine by in-tube extraction and gas chromatography – mass spectrometry (ITEX-GC-MS)

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Introduction: Ethylene glycol (EG) and methanol (MA) are commonly used anti-freeze agents and solvents that occasionally cause severe, even fatal poisonings, when ingested by accident. The dangerous nature of EG and MA is due to their toxic metabolites, mainly glycolic acid (GA) and formic acid (FA), respectively. Clinical diagnosis of poisoning is not straightforward, and this may end in delay of laboratory analysis and treatment. In post mortem investigation, the parent toxicants may not be detectable anymore due to long and intensive hospital treatment. The polar character of EG, GA and FA rules out analysis of these compounds among alcohols by ordinary head space GC methods.

Aim: The purpose of the work was to develop a quantitative analysis method for EG, GA and FA in autopsy blood and urine by head space ITEX-GC-MS. For logistic reasons, a prerequisite was using the same instrumentation and column as has been previously described for screening volatile organic compounds (VOC).

Methods: Sample volume was 0.5 g (0.5 ml) for both blood and urine. The extraction device was a CTC CombiPAL autosampler with ITEX and static headspace options. The GC-MS was an Agilent Technologies 6890N GC/5975 VL MS equipped with a PoraPlot Q HT column. Deuterated internal standards were used for EG and FA. Identification was based on full scan AMDIS spectra and retention time.

Results: A major finding was that hydroxylic and carboxylic compounds could be derivatised with dimethyl sulphate in biological material to form ethers and esters, respectively, making EG, GA and FA amenable to head space analysis. Blood samples were first precipitated with acetonitrile, whereas urine samples were used as such. The limit of quantification (LOQ) for EG and FA in blood was 0.01 and 0.03 g/l, respectively, and LOQ in urine was 0.03 and 0.05 g/l, respectively. GA was analysed semiquantitatively due to the lack of an appropriate internal standard. Expanded uncertainty of measurement for EG and FA in blood was 20% and 15%, respectively, and the values for urine were 13% and 14%, respectively. Case analysis showed that GA was always present in EG poisonings. In MA poisonings, FA was always present: the range of FA in blood was 0.1-1.1 g/l and FA/MA ratio was generally 0.3-0.4. However, considerable levels of FA were systematically detected in other cases, not related to MA poisoning. FA concentrations generally ranged from 0.1 to 0.2 mg/l in putrefied cases, while the highest value was 1.2 mg/l.

Conclusion: We have presented the first practical head space method for the analysis of EG, GA and FA in a forensic toxicology context. Derivatisation with dimethyl sulphate allows the determination of these compounds using the same ITEX-GC-MS instrumentation and column as for screening a wide range of VOCs. Caution is needed to interpret findings of FA for confirming MA poisoning, because FA is also found in other cases, especially in putrefied material.

Keywords: ethylene glycol, glycolic acid, formic acid, in-tube extraction, GC-MS

O13. Establishment of a simple and rapid determination method for a small amount of propofol in biological samples by gas chromatography-mass spectrometry

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Introduction: Propofol is a widely used intravenous agent for induction and maintenance of anaesthesia and for sedation in intensive care patients. However, the international literature is poor in reports dealing with propofol determination in solid tissue samples, especially at low concentrations.

Aim: To establish a simple and rapid method for the determination of a small amount of propofol in biological samples by gas chromatography-mass spectrometry (GC-MS).
Methods: 0.2 g of tissue sample (whole blood, brain, liver and adipose tissue) was homogenized in 1 ml of carbonate buffer (pH 11) and 50 ng of thymol as internal standard (IS) was added to the solution. The mixture was vortex-mixed with 100 ml of heptane, centrifuged and stored at -20 °C for 20 minutes. An aliquot of the supernatant was injected into GC-MS. Mass spectrometric detection of the alalyte was performed in the selected ion monitoring mode using the ions m/z 163 and 178 for propofol and m/z 135 and 150 for IS.
Results: Propofol and IS appeared at the time of 7.60 min and 7.09 min, respectively and no interfering peaks derived from biological samples were observed. The calibration curves were linear in the concentration range from 50 to 5000 ng/g in all tissues. The limit of detection was 2.5 ng/ml in blood, 5 ng/g in brain and liver, and 15 ng/g in adipose tissues. The CV values were within 11% for blood, 11% for brain, 4% for liver and 5% for adipose tissues when 50 ng/g of propofol were added. The absolute recoveries of propofol ranged from 25 to 30% in blood, brain and liver, and 10% in adipose tissues. The developed method was successfully applied to the analyses of the specimens from a forensic autopsy, in which the victim died after the misinjection of propofol. Propofol was detected in blood, brain, liver and adipose tissues, and its concentrations were 26.2 ng/ml, 129.6 ng/g, 10.4 ng/g and 2023 ng/g, respectively.
Conclusion: A simple and rapid determination method was established for a small amount of propofol in biological samples. It was successfully applied to the analyses of the specimens from a forensic autopsy.
Keywords: propofol, thymol, GC/MS

O14. Analysis of hair after contamination with blood containing cocaine and blood containing benzoylecgonine

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Introduction: In *post mortem* work, blood is a potential source of external contamination of hair. The hair can become contaminated by blood from an external injury or during the *post mortem* procedure. This can lead to false positive results and the misinterpretation of findings.
Aim: The present study was carried out to investigate the amount of drug absorbed into hair which has been contaminated with blood containing either cocaine or BE. Cocaine and BE were kept separate so that any breakdown of cocaine to BE could be monitored.
Methods: Solutions were prepared containing 0.05, 0.1, 0.2, 0.5 and 3.0 mg/mL of either cocaine or BE in human blood. These are the concentration ranges associated with recreational use of cocaine. Samples of approximately 3.2 g of drug-free hair were contaminated by soaking in the blood solutions for 5 minutes. They were then removed and left at room temperature. Approximately 0.5 g of hair was collected from each of the blood soaked hair samples at 6 hours, 1, 2, 4 and 7 days after contamination. As each hair sample was collected it was shampoo-washed in order to prevent further drug absorption. Hair samples were analysed in triplicate using a fully-validated method described previously. EME and cocaethylene were also measured in order to find out if cocaine or BE was breaking down to these compounds.
Results: The LOQ for cocaine, BE, EME and cocaethylene was 0.2 ng/mg hair. For hair contaminated with blood containing cocaine at 0.05, 0.1 and 0.2 mg/mL, cocaine was detected at all sampling times <LOQ while EME, BE and cocaethylene were not detected. For hair contaminated with blood containing cocaine at 0.5 mg/mL, cocaine was present in hair at all sampling times at approximately the same concentration (range: 0.48 +0.02 to 0.63 +0.01 ng/mg hair) and EME was detected in all samples <LOQ while BE and cocaethylene were not detected. For hair contaminated with blood containing cocaine at 3.0 mg/mL, cocaine was present at all sampling times at approximately the same concentration (range: 3.37 +0.06 to 4.16 +0.05 ng/mg hair), EME was detected <LOQ at 6 hours and >LOQ from 1 day onwards (range: 0.59 +0.03 to 0.99 +0.02 ng/mg hair) while BE and cocaethylene were detected <LOQ at all sampling times. For hair contaminated with blood containing BE at 0.05, 0.1 and 0.2 mg/mL, BE was not detected >LOQ at any of the sampling times. For hair contaminated with blood containing BE at 0.5 mg/mL, BE was present in hair at all sampling times (range: <LOQ to 0.58 +0.05 ng/mg hair). For hair contaminated with blood containing BE at 3.0 mg/mL, BE was present at all sampling times (range: 0.59 +0.04 to 1.35 +0.17 ng/mg hair). Cocaine, EME and cocaethylene were not detected in any hair samples contaminated with blood containing BE.
Conclusions: Both cocaine and BE were absorbed into hair in significant concentrations when the concentration in the blood was 0.5 mg/mL or greater, cocaine was more readily absorbed than BE. Cocaine broke down to EME (<LOQ) at 0.5 mg/mL and to EME (>LOQ) and BE (<LOD) at 3.0 mg/mL. When the blood concentration of cocaine was 0.5 mg/mL or less, there was no evidence of it breaking down to form BE. There was no evidence of the breakdown of BE. The absorption of drug into hair did not increase as the contamination period increased from 6 hours to 7 days.
Keywords: hair analysis, blood contamination, cocaine, EME, BE, cocaethylene

O15. The role of oxycodone metabolites in oxycodone related fatalities in the West of Scotland

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Introduction: In the United Kingdom, oxycodone is available on prescription in two forms, OxyContin (slow-release tablets) and OxyNorm (liquid/capsules). In Scotland, the number of prescriptions for oxycodone has risen by 430% since prescribing began in 2002.
Aim: The focus of this study was to review fatalities involving oxycodone in the West of Scotland using an LC-ESI-MS-MS method developed for the determination of oxycodone and N- and O-demethylated metabolites, noroxycodone and oxymorphone, in unhydrolysed *post mortem* specimens.
Methods: Deuterated internal standards were added to samples collected *post mortem* and analytes extracted using Bond Elut C18Ò cartridges. Separation was achieved using a Synergy Polar RP column (150 × 2.0 mm, 4 µm), gradient elution (mobile phase with (A) 10 mM ammonium formate, pH 3 and (B) acetonitrile), at a flow rate 0.3 ml/min using a Thermo-Finnigan LCQ Deca Plus instrument in the ESI SRM mode.
Results: The developed method for oxycodone and its metabolites noroxycodone and oxymorphone was linear over the concentration ranges 5 - 250 ng/mL and 50 - 5000 ng/ml with correlation coefficients (R2) greater than 0.999. Limits of detection and lower limits of quantification were 0.2 - 0.4 ng/mL and 1.0 - 1.2 ng/mL, respectively. Ten oxycodone positive *post mortem* cases were detected during the period July 2007-December 2008 in which nine deaths were drug related fatalities. Five of these were attributed solely to oxycodone intoxication and four cases to mixed drug intoxication. High levels of oxycodone in combination with low levels of noroxycodone and oxymorphone were identified in one case of suicide involving the deliberate ingestion of multiple tablets of OxyContin™. In four cases a number of undigested oxycodone tablets were identified in the stomach contents. Although there was overlap between blood oxycodone concentrations in deaths attributed to oxycodone only and poly-drug intoxication, the latter were associated with oxycodone concentrations less than 1 mg/L, while most cases in which oxycodone was the cause of death often had blood concentrations higher than 1 mg/L. The role of parent drug in oxycodone fatalities has been fully studied but the role of the metabolites noroxycodone and oxymorphone in oxycodone fatalities was investigated

in this report for the first time. Oxycodone was most commonly present in blood, urine and vitreous humor followed by noroxycodone. In some cases oxymorphone was not detected in blood but was found in urine. The ratio between parent drug and its N-demethylated metabolite is a useful tool for determining whether death occurred shortly after drug overdose ingestion or if it was delayed. It was found that the higher the ratio the shorter the elapsed time after ingestion before death occurred.

Conclusion: Oxycodone prescriptions have risen sharply in Scotland in recent years and the identification of ten oxycodone-related deaths in the past 18 months highlights the importance of including this drug in routine laboratory screening and confirmation procedures.

Keywords: Oxycodone, noroxycodone, oxymorphine, OxyContin™, LC-ESI-MS-MS

O16. Confession of a murder – Acquittal by a hair's breadth

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Introduction: In 2005, a young woman had been taken to a police station to have a blood sample taken, because she had shown signs of impairment of driving ability. Surprisingly, she did not confess intake of drugs but the murder of her father, committed 6 years ago. She testified that her mother had sedated the victim over several days till the point of complete defencelessness. Her mother urged her to strangle the victim to death using a sailing rope. They transported the corpse all the way down to the south of France, leaving it in the middle of nowhere. Consequently, French police was called in. They reported to have found some remains of a human being at that exact place. The remains (some bones and a skull) were sent to the Institute of Legal Medicine in Homburg for analysis. Because the mother denied sedation and murder of her husband, it was necessary to find some evidence for the daughter's story.

Aim: Sedative drugs should be detected in the remains in order to support the daughter's statement.

Methods: Some milligrams of mummified tissue that could be scraped off some bones were analyzed first by GC-MS after liquid-liquid extraction or solid phase extraction. A very small amount of hair that had been identified by DNA analysis as belonging to the victim was analyzed by LC-MS/MS after hydrolysis and solid-phase extraction.

Results: First, opipramol could be detected in mummified tissue by GC-MS. However, this finding was not suitable to support the daughter's statement, because the victim has had a corresponding prescription some month before his death. Fortunately, chlorprothixene could be detected additionally in the few hairs, that were available for analysis. Because of the high sensitivity of the LC-MS/MS technique even segmental analysis was possible, proving the exposition of the victim to chlorprothixene during the last weeks of his life. Finally, the judges were convinced that the statement of the daughter was true and the murders were sentenced to long imprisonment.

Conclusion: Highly sensitive hair analysis could help in solving an unusual murder case.

Keywords: Hair analysis, chlorprothixene, opipramol, GC-MS, LC-MS

O17. Analysis of benzodiazepines in blood and urine with automated disposable pipette extraction and HPLC/MS/MS

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Introduction: Disposable pipette extraction (DPX) is a novel solid-phase extraction device that uses loosely contained sorbent contained in a pipette tip. The main advantages of DPX technology are that the extractions are very rapid, negligible solvent waste is generated, and the extractions can be fully automated and coupled to chromatographic injections.

Aim: This study focuses on the automated extraction of reduced sample volumes coupled to LC/MS/MS to provide high throughput analysis of benzodiazepines in blood and urine specimens. The goal is to decrease the extraction time so that the extraction of one sample is completed during the chromatographic analysis of the previous sample of a sequence.

Methods: Using a GERSTEL MPS-2 instrument, the DPX extractions were performed in approximately 5 minutes using reversed phase (DPX-RP) and cation exchange (DPX-CX and DPX-WCX) mechanisms. Several different solvent systems were used to determine the most efficient means of introducing the eluent into the LC/MS/MS instrument (Agilent 6410 LC/MS/MS).

Results: Sample preparation was minimal and simply involved protein precipitation of blood with acetonitrile and enzymatic hydrolysis of urine specimens. All of the rest of the analysis was completely automated. Some modified applications of DPX combined the highly efficient extractions with filtrations to further simplify sample preparation and streamline the analysis for LC. The DPX extractions are performed in less than 6 minutes for all automated extractions, providing greater than 80% recovery for all benzodiazepines with less than 6% RSDs. Limits of detection were found to be less than 5 ng/mL for all of the benzodiazepines tested from both blood and urine specimens. The DPX extractions prove to remove potential matrix interferences and ion suppression, thereby providing high sensitivity

Conclusion: Automated DPX using the GERSTEL MPS-2 with Agilent 6410 LC/MS/MS provides rapid and high sensitive detection of benzodiazepines in blood and urine specimens.

O18. Rapid quantification of tilidine, nortilidine and naloxone in urine by LC-ESI-MS/MS

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Introduction: Due to the increasing abuse of the opioid tilidine and the lack of a specific immunoassay, we have developed an analytical method for the quantitative determination of tilidine, nortilidine and naloxone in urine. Tilidine is a prodrug which is hepatically metabolized to its active form nortilidine. The latter is an agonist at μ-opioid receptors mediating its strong analgesic effect. In commercially available preparations tilidine is combined with the opiate antagonist naloxone to prevent the abuse of tilidine in higher doses. Naloxone does not influence the analgesic effect at therapeutic doses since it is rapidly degraded into inactive metabolites during the hepatic first pass. More than 3500 urine samples were screened for tilidine, nortilidine and naloxone. In addition, a single oral dose of Valoron®N solution (50 mg tilidine-HCl and 4 mg naloxone-HCl) was given to healthy volunteers to determine the concentration-time profile of the opioides. A commercially available opiate immunoassay was challenged with tilidine, nortilidine and naloxone to test for positive results.

Aim: The purpose of this study was the development of a sensitive and validated method for the quantification of tilidine, nortilidine and naloxone in urine samples. In two self-experiments, the drug detection windows and analyte concentrations were investigated in two self-experiments.

Methods: Urine samples were extracted with TRIS-buffer (pH 9) and 1-chlorobutane containing the internal standard phencyclidine-D5 (c=100 μg/L). The chromatographic separation was achieved within 5 minutes run time on a Varian Pursuit PFP column (5 μm, 150 × 3.0 mm) using a gradient consisting of a mixture of methanol, formic acid and ammonium acetate (flow rate: 0.60 mL/min). The ESI-MS/MS was performed via MRM mode on a 3200 QTrap using the following transitions (m/z): 274.4 → 155.1 and 274.4 → 115.0 for tilidine, 260.2 → 155.1 and 260.2 → 115.0 for nortilidine, 328.2 → 212.1 and 328.2 → 253.2 for naloxone and 249.3 → 96.1 for phencyclidine-D5.

Results: Analyses of spiked blank urine resulted in a LLOQ of 1.0 µg/L followed by a linear calibration range to 100 µg/L for each analyte (r2>0.998). A total number of 3599 urine samples were analyzed achieving 111 positive results (3.1%). Only a minority of these positive samples (12.6%) also revealed naloxone presence. The analysis of urine samples of two male volunteers after the administration of a single oral dose of 50 mg tilidine-HCl and 4 mg naloxone-HCl showed maximum concentrations of 322 µg/L for tilidine, 4035 µg/L for nortilidine and 2.5 µg/L for naloxone. In order to test the interference with commercially available immunoassays for drug detection, blank urines spiked with tilidine, nortilidine or naloxone (c = 10 mg/L) were screened for amphetamines, benzodiazepines, cocaine, opiates and THC. Only the naloxone-spiked sample was positive for opiates due to its structural similarity to morphine.

Conclusion: An LC-MS/MS method for the highly specific and sensitive quantification of tilidine, nortilidine and naloxone in urine has been developed. The LLOQ of 1.0 µg/L allowed the detection of a single dose of Valoron® N solution up to 5 days after administration. As expected authentic samples showed no positive opiate result.

Keywords: tilidine, nortilidine, naloxone, urine analysis

O19. Capillary liquid chromatography with nano-electrospray ionization tandem mass spectrometry for ultra-sensitive analysis of drugs in hair

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Introduction: For many forensic applications it is highly desirable that analytical approaches are as sensitive as possible. Capillary liquid chromatography (cLC) columns (20 µm) have very low internal volumes and use nL/min flow rates. These dimensions lead to increased analyte sensitivity due to increased analyte concentration on column. The coupling of cLC to mass spectrometry (MS) using a nano-electrospray ionization (nanoESI) source that can work with nL flow rates also brings sensitivity gains. This is due to the small droplets that are formed in nanoESI leading to more efficient solvent evaporation and reduced ion-suppression caused by an increase in droplet surface area and a reduction in analyte diffusion time. By applying cLC-nanoESI tandem MS (MS/MS) large increases in sensitivity can be achieved.

Aim: To demonstrate the large increases in sensitivity that can be achieved by using cLC-nanoESI-MS/MS for the detection of drugs in hair. Using ketamine as a model analyte, a comparison of the approach was made to conventional LC-MS/MS typically used in analytical toxicology.

Methods: Samples of hair were taken from volunteers that had been administered low doses of ketamine. A 'pencil thickness' of hair (5 mm) was taken from the volunteers seven weeks post administration and the drugs were extracted by first grinding the hair in a ball mill and then using a solvent extraction. The cLC columns with integrated nanoESI emitters were manufactured and packed in-house and were coupled to a triple quadrupole mass spectrometer that could be fitted with both a regular ESI source and a nanoESI source. A nanoflow LC pump was used to deliver a low flow gradient for analytical separation on the cLC column and 1 µL of sample was injected.

Results: The cLC-nanoESI approach showed significant improvements in sensitivity in comparison to conventional LC-MS on the triple quadrupole instrument. Ketamine was not detected in the hair extracts but its primary metabolite norketamine and a secondary major metabolite dehydronorketamine was detected in all samples. We observed a relative increase in sensitivity of at least 100 fold when using the cLC-nanoESI approach versus conventional LC-MS.

Conclusions: Using cLC-nanoESI coupled to tandem mass spectrometry brings great increases in sensitivity for toxicological analysis. This is particularly useful for difficult matrices such as hair where the expected concentrations of drugs are much lower then those found in traditional samples such as blood and urine.

Keywords: nano-electrospray, capillary liquid chromatography, hair analysis, ketamine

O20. Detection and identification of 700 drugs by multi target screening (MTS) with a QTrap 3200 LC-MS/MS and library searching

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Introduciton: A library with ESI MS/MS spectra of 1250 compounds has been developed using a QTrap 3200 tandem-mass spectrometer (Applied Biosystems) with a turbo ionspray source. After standardisation of a chromatographic system using a 50 mm × 2.1 mm Allure PFP column (Restek), the library has been used for the identification of drugs and metabolites in urine and serum/blood samples using a "multi target" general-unknown screening approach.

Aim: Development and application of a drug screening procedure with LC-MS/MS for general unknown screening.

Methods: Retention times of 700 compounds have been determined and transitions for each compound were selected by a "scheduled" survey-MRM scan, followed by an information dependent acquisition (IDA) using the sensitive enhanced product ion scan of the Qtrap hybrid instrument. A library search was performed for compound identification. Due to the selection of MRM transitions, the method is called multi target screening [1], now covering 700 compounds in a single LC-run (drugs of abuse, psychoactive drugs and many others). Automation of data exploration has been performed.

Results: Standardisation of the procedure has been performed for its applicability in different laboratories, using a reference standard test mixture ("suitability test mix"), and also internal deuterated standards for semiquantitative analysis for several drugs. First applications of this procedure have been developed for the detection and identification of drugs of abuse and drugs for substitution (opiates, amphetamines, cocaine, LSD, cannabinoids, buprenorphine, and methadone), psychopharmaceuticals (benzodiazepines, hypnotics, antidepressants, neuroleptics) and pain relief drugs. Urine samples of drug abusers, from clinical and forensic cases (material from autopsy) have been investigated, with the aim of testing the reproducibility and robustness of the system, especially in terms of comparison of different sample preparation procedures (dilution 1:10 and 1:3 [v/v], or extraction with chlorobutane at pH 9) and matrix effects. With the use of the internal standards, the system could be used for drug identification as it has been demonstrated by GC/MS and HPLC-DAD analysis performed in parallel. The optimised method allows the detection and identification of a great variety of compounds within one analytical run of 15 min using a gradient elution with steadily increasing flow rate. Limits of detection were in many cases lower than those of classical immunoassays (amphetamines, opiates, benzodiazepines, cocaine-metabolite) and will be reported.

Conclusions: The application of this screening method is in the fields of clinical toxicology, psychiatry (antidepressants and other psychoactive drugs), and forensic toxicology (drugs and driving, workplace drug testing, oral fluid analysis, drug facilitated sexual assault) – whenever different classes of drugs are relevant.

Reference: [1] Mueller CA, Weinmann W, Dresen S, Schreiber A, Gergov M, Rapid Commun Mass Spectrom. 2005; 19(10) :1332-1338.

Keywords: multi-target screening, MS/MS library, enhanced product ion scan.

O21. Construction of calibration locking databases for rapid and reliable drug screening by gas chromatography-mass spectrometry

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Introduction: Analysis of drugs and/or poisons in biological samples is an important routine task in clinical and forensic toxicology, and GC-MS using commercially available mass spectra libraries is often used for that purpose. However this kind of approach requires considerable time and experienced analyst for data analysis, because these libraries mainly give information for mass spectra.

Aim: The purpose of our work was first, to construct unique calibration-locking databases for rapid and semi quantitative drug screening by GC-MS without using standard compounds, and second, to examine the applicability of our new screening procedure using constructed databases by analyzing spiked whole blood samples and forensic autopsy samples.

Methods: In addition to the free-drug database of 127 drugs, a drug database with acetylating reagents of 156 drugs was constructed to increase the number of detectable compounds in the analysis by GC-MS. Each drug was registered with parameters, such as mass spectrum, retention time (RT), qualifier ion/target ion (QT) percentage and calibration curve using a novel GC-MS software NAGINATATM. Diazepam-d5 was used as internal standard for constructing each calibration curve. The applicability of the constructed database was examined by analyzing whole blood samples spiked with 40 drugs most commonly encountered in toxicological cases in Japan; the drugs in blood were extracted using an enhanced polymer column FocusTM, and subjected to GC-MS after incubating with acetylating reagents, and screened by the drug database.

Results: Among 40 drugs examined, 38 and 30 drugs were successfully identified at the level of 1 and 0.1 mg/ml, respectively without using standard compounds. The time required for data analysis was less than 1 min, and semi-quantitative data were also obtained simultaneously. Analyses of whole blood and urine samples of forensic autopsy cases also gave reliable results.

Conclusion: Since our new screening procedure using constructed databases can produce the same results in every GC-MS instrument without using standard substances, we can recommend this approach as a useful tool for clinical and forensic toxicological screening.

Keywords: calibration-locking database, systematic toxicological analysis, enhanced polymer column, GC-MS, acetylation, NAGINATATM

O22. Use of the biochip array technology in forensic toxicology - comparison with Microplate EIA, GC-MS and LC-MS

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Introduction: Immunoassays are usually used in forensic toxicology in order to differentiate between negative and presumptively positive samples. A new immunoassay technique employing the so called biochip array technology has been introduced some years ago. Multiple specific antibodies are attached at pre-defined sites on the surface of the biochip. Recently, the "Drugs of Abuse II" biochip has been released.

Aim: The aim of the presented study was to compare the results of the biochip system with the usually used EIA, GC-MS and LC-MS techniques.

Methods: Routine authentic serum samples were tested. The Randox (Randox, Crumlin, UK) evidence investigator biochip reader was used for evaluation of the "Drugs of Abuse I" (amphetamines, methamphetamines, barbiturates, benzodiazepines (BZD) 1, BZD 2, cocaine metabolite, methadone, opiates, phencyclidine, cannabinoids) and the "Drugs of Abuse II" (ketamine, oxycodone, buprenorphine, fentanyl, propoxyphene, methaqualone, MDMA, LSD) biochips. The corresponding Microplate EIAs (Orasure, Bethlehem, PA, USA and NAL, Pormona, CA, USA) were analyzed by a BioRad Coda Automated EIA analyzer (BioRad, Hercules, CA, USA). GC-MS and/or LC-MS confirmation of immunoassay positive and negative samples was achieved using routine methods.

Results: Over 800 single immunoassays could be evaluated: amphetamines (n=98), methamphetamine (n=75), benzodiazepines (n=24), methadone (n=72), opiates (n=71), cocaine metabolite (n=75), cannabinoids (n=73), ketamine (n=110), buprenorphine (n=51), fentanyl (n=73), LSD (n=13), MDMA (n=50) and oxycodone (n=25). Cut-off values were adjusted to meet forensic demands. Bayesian analysis of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) was used to determine the parameters: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Most of the parameters were in the 90 percent range compared to the LC-MS or GC-MS confirmation results and thus on par with the microplate EIA assay.

Conclusion: The Randox system performed quite well. Handling was simple. Very little sample volume is needed (only 25 to 100 µL for all 9 assays on biochip). The biochip array technology could be an alternative to the usual microplate enzyme immunoassays.

Keywords: biochip array technology, EIA, GC-MS, LC-MS, Bayesian analysis

O23. Evolution of liquid chromatography: current and future trends

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Introduction: These last years, a strong development has emerged in liquid chromatography (LC) in terms of chromatographic supports and instrumentations to achieve fast and efficient separations. Various analytical strategies have been reported, such as the use of monolithic supports, high temperature liquid chromatography or sub-2 µm particles working under very high pressure (UHPLC). Among the proposed approaches, it has been demonstrated that, in many cases, UHPLC was a particularly promising one. Beside its performances, an additional reason for the success of this technique was related to the easy transfer of existing LC methods toward UHPLC, using basic rules of chromatography.

Aim: The purpose of this work was to demonstrate that UHPLC can be used to significantly increase the analysis throughput and thus efficiently manage a large number of samples. This is particularly beneficial in the pharmaceutical and toxicological fields. Alternatively, it was also possible to generate very high resolution for metabolite profiling in more complex samples such as plant extracts and biological fluids.

Results: The potential of UHPLC was evidenced using both theoretical models and experimental results. A strong emphasis was also made on the possibilities offered by UHPLC at high mobile phase temperature, up to 90 °C (*i.e.* HT-UHPLC). Indeed, raising the temperature induces a significant reduction in the mobile phase viscosity, allowing the application of higher flow rates at a given backpressure. Separations of drugs, doping agents and complex plant extracts were carried out to highlight advantages and issues of this approach.

Conclusion: The different applications investigated have demonstrated that UHPLC with sub-2 µm particles columns is a very promising tool in the field of analytical sciences. In addition, the application of elevated temperatures is of utmost interest to further enhance chromatographic performance.

O24. Drug screening in whole blood with ultra performance liquid chromatography tandem mass spectrometry

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Introduction: Screening of drugs of abuse and psychoactive medicinal drugs is important both in driving under the influence and autopsy cases. At the Norwegian Institute of Public Health these substances have been screened with a combination of immunoassay analysis (EMIT) and LC-MS. One specific and sensitive screening method, where the repertoire can be changed relatively easy in accordance with trends in drug abuse and prescription practise, was however desirable.

Aim: The purpose of our work was to develop a fast and specific screening method for drugs of abuse and psychoactive medicinal drugs using ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS).

Methods: Drugs were extracted from 500 ml of whole blood by immobilized liquid-liquid extraction (LLE) with ethylacetate/heptane (4:1) using ChemElut columns. Chromatographic separation was achieved using an Aquity UPLC HSS T3 column (2.1 × 100, 1.8 µm). The mobile phase consisted of ammonium formate pH=3.1 (A) and methanol (B), with a gradient from 10% B to 90% B. Mass detection was performed by positive ion mode electrospray tandem mass spectrometry and included the following drugs/metabolites: morphine, codeine, buprenorphine, ethylmorphine, oxycodone, methadone, fentanyl, cocaine, benzoylecgonine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), cathinone, methylphenidate, Δ-9-tetrahydrocannabinol (THC), alprazolam, bromazepam, clonazepam, diazepam, N-desmethyldiazepam, fenazepam, flunitrazepam, lorazepam, midazolam, nitrazepam, oxazepam, zopiclone, zolpidem, carisoprodol, and meprobamate and internal standards: morphine-d3, codeine-d6, amphetamine-d11, methamphetamine-11, MDMA-d5, benzoylecgonine-d8, cocaine-d3, clonazepam-d4, buprenorphine-d4, N-desmetyldiazepam-d5, diazepam-d5, methadone-d9 and THC-d3.

Results: Screening of 30 drugs was performed with a run time of 8 minutes. Within- and between-day relative CVs varied from 1.2%-68% and 1.6%-101%, respectively. Extraction recoveries (average of 3 concentration levels) were >50% except for morphine (47%), catinone (32%), benzoylecgonine (2%) and THC (30%). LOQ varied between 0.0003 and 2 µmol/L, depending on the drug. Matrixeffects varied between 73 and 166% corrected for internal standard (88 and 2382% uncorrected).

Conclusion: A fast and specific method covering drugs of abuse and psychoactive medicinal drugs commonly found in driving under the influence samples and autopsy samples in Norway has been developed and validated.

Keywords: UPLC-MS/MS, drugs of abuse, psychoactive medicinal drugs, whole blood, LLE

O25. Ketamine and norketamine detection in hair by molecularly imprinted solid phase extraction (MISPE) *versus* solid phase extraction (SPE) prior to LC-/MS/MS analysis

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Background: This preliminary study was a comparison of validated MISPE and conventional SPE methods coupled to LC-MS/MS for the analysis of ketamine and norketamine in hair samples from living subjects. An anti-ketamine imprinted polymer was synthesized and used as MISPE sorbent; the polymer was capable of rebinding ketamine or structurally similar molecules, in a strong and selective manner.

Samples: Blank hair samples for method development and validation were from volunteers in the laboratory. The method application was tested on real hair samples collected with informed consent from four chronic female ketamine users at a drug misuse prevention centre in Malaysia.

Method: Hair samples were decontaminated with 0.1% aqueous sodium dodecyl sulfate (SDS), deionised water and dichloromethane, then air dried. 10 ± 0.1 mg samples plus 50 ng deuterated ketamine and norketamine internal standards were incubated for 18 hours at 45 ºC in 1.5 mL 0.1 M phosphate buffer pH 5.0 and subsequently extracted by SPE and MISPE followed by LC-MS/MS analysis. Clean Screen® (ZSDAU020) cartridges were used for SPE and compared with MISPE.

Results: MISPE and SPE coupled with LC-MS/MS both gave methods which were linear from 0-10 ng/ mg hair with R2 better than 0.99 for both ketamine and norketamine. For MISPE, the LODs for ketamine and norketamine were 0.10 ng/mg hair and LLOQs were 0.4 and 0.5 ng/mg hair while for SPE, LODs were 0.5 ng/mg for ketamine and norketamine and LLOQs were 0.9 and 1.8 ng/mg. The recoveries were above 85% for both analytes and methods. The average ketamine and norketamine intra- and inter-batch imprecision were <5% for the pooled hair sample on both methods. MISPE showed low matrix effects in hair during LC-MS/MS analysis; ketamine (ion suppression, -6.8%) and norketamine (ion enhancement + 0.2%) whereas very significant values were obtained with the SPE method for the two analytes. MISPE successfully detected ketamine and norketamine in all four hair samples with ketamine levels ranging from 0.2-5.7 ng/mg and norketamine from <0.5 ng/mg to 1.2 ng/mg. SPE detected ketamine at concentrations ranging from 0.5 – 6.7 ng/mg but norketamine was not detected in two out of the four samples and was less than LLOQ in the other 2 samples.

Conclusion: MISPE coupled with LC-MS/MS demonstrated good selectivity and sensitivity for ketamine and norketamine in hair. SPE coupled with LC-MS/MS showed higher selectivity to ketamine than MISPE but less sensitivity for norketamine. High matrix effects in the SPE method might have interfered with the LC-MS/MS analysis and caused less sensitivity for norketamine detection in hair.

Keywords: MIPs, SPE, ketamine, norketamine, hair, LC-MS/MS

O26. MRM³ – a sophisticated tool for sensitive and selective detection of THC carboxylic acid directly from hair

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Introduction: The detection of THC carboxylic acid (THC-COOH) is currently of much Bioanalytical interest. More commonly known as Cannabis, it is one of the most popular misused illicit drugs of our times. To date several LC-MS/MS methods have been published showing sufficient sensitivity for the determination of the major THC metabolite measured in bodily fluids such as urine and serum/blood. But the lifetime of this substance in the above mentioned matrices is much shorter when compared directly to the longer lifetime of this metabolite in hair. Therefore making the verification of regular THC consumption inauspicious; thus requiring a reproducible method that is more effective for the analysis of this metabolite (residue) found in hair.

Aims: Difficulties in a selective analysis arise due to the complexities of the matrix. Hair contains various substances similar in structure and mass to THC-COOH, for example fatty acids. This causes interference with the analyte´s ion transitions in the normal MRM mode of detection. Due to the structural and physical affinity of fatty acids, classical sample preparation does not provide a significant improvement in the selectivity of the overall

method. Implementing a highly selective MS experiment offers a simple and effective method/solution to improve the sensitivity in the detection of analytes in difficult matrices.

Methods: A simple, fast, sensitive and highly selective LC/MS/MS/MS, LC/MRM3, method for the detection of THC-COOH from hair was developed using an Agilent 1200 HPLC coupled to the new AB Sciex QTrap® 5500. Total run time of the gradient was 8 minutes, including 1.5 minutes re-equilibration time for the column.

The MRM³ transition 343.1 / 299.2 / 245.1 was implemented for quantification and qualification of THC-COOH. The deuterated analogue THC-COOH-D3 was used as an internal standard. It was monitored using the MRM³ transition 346.1 / 302.2 / 248.1.

Results: The lowest concentrated standard solution, 20 pg/ml THC-COOH, could be easily determined, corresponding to a concentration of 0.1 pg/mg THC-COOH in hair. This was also achievable in real sample matrix. The linear range of the MRM3 quantification was comparable to the common MRM quantification methods with coefficients of correlation higher than 0.997.

Conclusions: The results demonstrate that by using a strategic and innovative MS experiment for the determination of THC-COOH, provides a plausible solution. Implementation of MRM3 methods not only eliminate matrix interference but also improve the overall quality of the analysis. This technique improves significantly both the sensitivity and selectivity in the detection of analytes in difficult and complex matrices.

Keywords: THC-COOH, cannabis, hair, analysis, LC-MS/MS/MS, MRM³

O27. Towards genuine identification without reference standards: application of fragmentation prediction in designer drug analysis

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Introduction: The limited availability of reference standards in designer drug and metabolite analysis result in multi-phased and complex procedures in identification of unknowns by traditional approaches. However, the combination of accurate mass measurement and current in silico tools provide efficient means for identification without reference standards, based on fragmentation prediction and accurate mass analysis of experimental data, as recently demonstrated in a study presenting quetiapine metabolite identification [1].

Aim: The aim of this study was to identify designer drugs in an authentic autopsy urine sample without reference standards, and prove the reliability of the approach by using the appropriate standard.

Methods and case description. The LC/TOFMS routine screening analysis used in the authors' laboratory includes approximately 850 compounds, including a representative selection of designer drugs. The analysis of a urine sample for case 775/09 indicated the presence of 23 different drugs and metabolites, for which retention time information was available. In addition, a high abundance signal was acquired for mass corresponding to fluoroamphetamine molecular formula. After ISCID (in-source collision induced dissociation) analysis the experimental data was compared to fragment pattern predicted by ACD/MS Fragmenter 11.01 software and information provided by Bruker DataAnalysis 4.0 SmartFormula3D software.

Results: In ISCID analysis three fragments were observed for fluoroamphetamine protonated molecule of 154.1027 Da. The measured masses were 137.0768, 109.04687 and 91.0540 Da. ACD/MS Fragmenter predicted the first two to correspond to cleavage of the amino (M+H – NH3) and ethyl amino groups (M+H – C2H7N). In addition, SmartFormula3D indicated additional F cleavage for the third ion (M+H – C2H7NF). The mass accuracies were -0.7, -2.0 and 0.3 mDa, respectively. HMMA, a MDMA metabolite and a designer drug as such, was also identified in the sample in the routine screening analysis. In ISCID analysis three potential fragments were observed, with measured masses of 165.0909, 137.0607 and 133.0667 Da, predicted as (M+H – CH5N), (M+H –C2H5N) and (M+H – C2H9NO) by ACD/MS Fragmenter software and SmartFormula3D. The mass accuracies were 0.1, -1.0 and -1.9 mDa, respectively. The HMMA fragmentation was confirmed by corresponding ISCID analysis of the reference standard. An additional confirmation of the presence of fluoroamphetamine was performed by GC/MS analysis against a commercial designer drug library.

Conclusions: The reliability of identification by accurate mass fragmental and isotopic pattern analysis was proven to be possible and reliable, exemplified by two designer drugs. Accurate mass data combined with current in silico tools facilitates spectral interpretation remarkably, and enables retention time assignment without reference standard. Automation of the process will provide means for screening/confirmation analysis in a single run.

Reference: [1] Pelander A, Tyrkkö E, Ojanperä I. Rapid Commun Mass Spectrom. 2009; 23(4): 506-514.

Keywords: in silico, fragmentation prediction, LC/TOFMS

O28. Simultaneous screening for and determination of 128 date-rape drugs in urine by gas chromatography-electron ionisation-mass spectrometry

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Introduction: Date-rape drugs are used for the purpose of "drugging" unsuspected victims and raping or robbing them while under the influence of the drug. Because of the wide variety of substances used for criminal purposes, their low concentrations and, often, a long time delay between the alleged crime and clinical examination screening analysis of urine samples collected from the crime victims for presence of these drugs was performed rarely until recently. Detection of a drug used to facilitate sexual assault in biological fluid can be very important evidence of a committed crime.

Aim: The purpose of this study was to develop a simple GC-EI-MS screening procedure for date-rape drugs in urine.

Methods: Target analytes were isolated from 2-mL of urine sample by SPE using Oasis HLB 3 cc (60 mg) colums. Methanol and isopropanol (3:1, v/v) were used as an elution mixture. Extracts were derivatized with BSTFA+1%TMCS at 60 °C and after cooling reconstituted with 60 µL of ethyl acetate:acetonitrile (1:1, v/v) mixture. Analyses were carried out using Trace GC Ultra/Polaris Q GC-MS operating in EI mode. Gas chromatograph oven was working in gradient temperature and injector in split mode. Mass spectra were registered in the range of 45-650 amu. Separation was performed on an Rtx-5 MS column (30 m × 0.25 mm × 0.25 µm). Total analysis time was 25.9 min. Detection of all compounds was based on full mass spectra and for each compound one ion was chosen for further quantification. For all drugs up to 7-points calibration curves were prepared.

Results: The GC-EI-MS method allowed the simultaneous screening, detection and quantification of the 128 compounds from different chemical groups (number of compounds): opioids (20), amphetamines (11), GHB and related (3), hallucinogens (9), benzodiazepines (18), antihistamines (9), antidepressants (14), selective serotonin-reuptake inhibitors (4), antipsychotics (7), barbiturates (7), other sedatives (5), muscle relaxants (2) and other drugs (19). No interfering peaks were observed in the extracts of ten blank urine samples. The LODs with a S/N≥3 were determined between 0.05 to 20 µg/mL for respective drugs. The assay was linear from LOQs (S/N≥10) up to 20-100 mg/mL. Recoveries ranged from 27 to 112% at 1 mg/mL for 20 compounds. Total precision (combination of within- and between-day processes) lay within the required limits of ≥15%RSD. Linear

regression correlation coefficients of the calibration curves were ≥ 0.96. Reconstituted extracts were stable for a period of more than 24 h at room temperature or for a week at +40 °C. The procedure can be easily expanded for more substances.

Conclusion: Developed method appeared to be suitable for screening for the target date-rape drugs. The procedure was successfully applied to the analysis of authentic urine samples collected from victims of rapes in routine casework.

Keywords: drug-facilitated sexual assault, screening, determination, GC-EI-MS

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O29. Comparison of rapid LC-MS/MS screening with conventional GC-MS screening in forensic toxicology

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Introduction: LC-MS/MS is gaining broader utility in forensic toxicology due to increasing sensitivity and selectivity of modern instruments. In addition, the ability to monitor multiple analytes in a single experiment allows us to apply the technique for rapid "overnight" screening of blood specimens for most drugs relevant to forensic toxicology. This study compared the detectability and reliability LC-MS/MS targeted screening with conventional GC-MS screening.

Methods: Forty extracts of *post mortem* and other forensic blood samples were obtained from treatment of blood basified to pH 9.2 with butyl chloride as used routinely in this laboratory. LC-MS/MS experiments utilised 0.1 mL blood and 1 mL butyl chloride. Eluant reconstituted extracts (100 µL) were applied to a model 3200 Q-Trap tandem LC-MS (ABI) with an Eclipse XBD 5 µm 4.6 × 150 mm C18 column (Agilent) as described previously [1]. Data generation and processing was automated using two MRM transitions per analyte and with retention time matching of over 100 substances. GC-MS experiments used 1 mL of blood and 6 mL butyl chloride. Methanol reconstituted extracts (2 µL) were run on a 25 m Ultra-2 0.5 µm film (0.32 mm i.d.) capillary column on a model 5975 GC-MS in full scan mode fitted also with a nitrogen phosphorous detector [2].

Results and Discussion: The LC-MS/MS method overall had a much lower LOD than the GC-MS procedure even with use of 0.1 mL sample volumes and with the assistance of macros was able to provide a result the following morning for reporting to clients after an overnight run. Drugs typically difficult to detect by underivatized GC-MS such as benzodiazepines and their metabolites, zopiclone, high potency anti-psychotics and morphine were readily detectable by tandem LC-MS. 6-Acetyl morphine was detected in some cases by LC-MS/MS in persons using heroin that was typically only detected previously using urine. The tandem LC-MS procedure was also capable of detecting a series of amphetamines, and cocaine and its metabolites. In all cases an approximate concentration was provided by a one-point daily calibration. A review of over 1500 *post mortem* cases completed by the laboratory indicated that the tandem LC-MS method was capable of detecting relevant drugs in 99% of Victorian cases, a much higher rate than GC-MS by itself (90%) when no other tests were conducted and when no particular information on drug usage was available. This has allowed the use of targeted LC-MS/MS screening to replace GC-MS screening without significantly sacrificing the "general unknown screening" capability of GC-MS and allowing much quicker turn-around of cases.

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O30. Drug hair analysis by liquid chromatography–high resolution mass spectrometry in an Orbitrap

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Introduction: Excellent mass resolving power and mass accuracies are the characteristics of Fourier-transform mass spectrometry (FTMS). Recently, an electrostatic trap with FTMS features (Orbitrap) has been developed. Coupled with a linear trap, the Orbitrap has proven to be an excellent analytical tool for low and high mass biomolecules, capable of performing both high resolution mass spectrometry (HRMS) and multiple mass spectrometry experiments (HRMS-MS).

Aim: The purpose of our work was to investigate on the power of full scan HRMS and HRMS-MS for the qualitative and quantitative detection of toxicological compounds and their metabolites in complex matrices, and to discuss the ability to perform highly selective quantitative analysis while simultaneously characterizing untargeted metabolites. Two methods were developed and applied, the former devoted to the documentation of gestational exposure to the antidepressant drug Venlafaxine (VEN) by neonatal hair analysis, the latter dedicated to the simultaneous detection of 26 analytes from different classes (opiates, cocaine, amphetamines, benzodiazepines, antidepressants, hallucinogens) in 2.5 mg of hair.

Methods: Both methods entailed a rapid (5 min) extraction procedure consisting of shaking 2.5 mg pulverized hair in a polypropylene vial in the presence of an acidic solution (water/acetonitrile/trifluoroacetic acid) containing the internal standards, and a stainless steel bullet. Obtained supernatants were submitted to analysis by LC-HRMS or LC-HRMS-MS in an Orbitrap mass spectrometer operating with a target mass resolution of 60,000 (FWHM as defined at m/z 400) in positive ion electrospray ionization mode. Gradient elution on an Atlantis T3 column was realized.

Results: The extraction procedure provided a drastic time reduction with respect to more conventional methods entailing sequential pulverization, overnight hydrolysis, solid-phase and/or liquid-liquid extraction steps. No significant differences in terms of relative recovery were observed. The method for VEN exhibited a linear range of 0.2-25 ng/mg, a quantification limit (LOQ) of 0.2 ng/mg, relative standard deviations in the range 0.7-1.4% (intra-assay) and 2.9-5.9% (inter-assay) and accuracies (as% relative error) in the range -9 to + 2%. The utilization of HRMS in full-scan mode allowed the determination of four VEN metabolites, namely O-desmethylvenlafaxine, N-desmethylvenlafaxine, N,N-didesmethylvenlafaxine and N,O-didesmethylvenlafaxine by means of retrospective screening in hair samples of two newborn twin sisters. HRMS-MS experiments were essential for their correct identification. The method for the 26 analytes yielded LOQs in the range 0.1 – 0.5 ng/mg. Calibration by linear regression analysis gave R2 from 0.9971 to 0.9999. Mean relative errors, calculated at three concentration levels, ranged from 3 to 19%. Precision, at concentrations higher than the LOQs, was always less than 10 as% relative standard deviation. The method was successfully applied to the analysis of hair samples in cases of clinical or forensic toxicology interest.

Conclusion: The Orbitrap is able to provide sensitivity, selectivity, dynamic range, and scan speed suitable for quantitative determinations of drugs in tiny amounts of hair. Full-scan HRMS acquisition eliminates the need for compound optimization in multi-analyte methods, while enabling the detection of metabolites and other non-drug-related endogenous components by a posteriori reconstructing the desired ion chromatogram. However, HRMS-MS proved to be essential for correct identification of untargeted metabolites.

Keywords: LC-HRMS, Orbitrap, hair analysis, venlafaxine, drugs of abuse

O31. Development of mass spectrometric method for identifying novel enzymes targeted by anti-cholinesterase organophosphorus compounds using shotgun proteome strategies

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Introduction: The organophosphorus pesticides are widely used to control pests all over the world. In addition, the chemical warfare nerve agents such as sarin have the same chemical frame as the organophosphorus pesticides. The mechanism of that toxicity is based on the inhibition of nervous acetylcholineseterase activity. In general, it is not doubtful that the main target of the organophosphorus poisoning is above-mentioned nervous acetylcholinesetrase, but it is known that the activities of other enzymes such as NTE (neuropathy target esterase) are inhibited by these compounds, leading to toxicity. It is expected that there are unknown target enzymes that take part in organophosphorus compound toxicity.

Aim: The aim of this study is to establish the analytical method for organophosphorus compound-combined peptides from purified model enzymes (human serum butyrylcholineserase (BuChE) and bovine a-chymotrypsin) as the first stage to identify novel target enzymes.

Methods: Purified BuChE and commercial a-chymotrypsin were reacted with diisopropylfluorophosphate (DFP) in phosphate buffer (pH 6.8) to completely inhibit the hydrolytic activity. The inhibited enzymes were reduced, cysteine-alkylated, and digested by proteases. The produced peptides were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using LTQ Orbitrap XL (Thermo Fisher Scientific). The peptide fragments reacted with organophosphorus compound were detected by the LC-MSn analysis with neutral loss measurement. Furthermore, the amino acid sequences of the organophosphorus combined peptides were characterized by searching the database using BioworksTM software.

Results: In the LC-MS/MS analysis of both a-chymotrypsin and BuChE digests using neutral loss measurement, the DFP-combined peptides were specifically detected. To improve specificity, it was effective to set the tolerance to make neutral loss mass defect narrow. As a result of the database searching, several peptides that combined with DFP were listed as candidates, and the binding positions of DFP were identified by comparing the obtained MS3 spectra with the deduced ones. For a-chymotrypsin and BuChE, the DFP combined peptides "IKDAMICAGASGVSSCMGDS*GGPLVCK" and "GES*AGAASVSL" were identified, respectively.

Conclusion: The analytical method for identifying the organophosphorus compound-combined peptides was established. By adopting this method, discovery of novel organophosphorus compound target enzymes will be accelerated.

Keywords: LC-MS, protease digestion, anti-cholinesterase insecticides, nerve gases, neutral loss measurement

O32. Radiological detection of dissolved cocaine

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Introduction: Smuggling dissolved drugs, especially cocaine, in bottled liquids is a problem at borders nowadays. Common fluoroscopy of packages at the border cannot detect contaminated liquids. To find a dissolved drug, an immunological test using a drug-test panel has to be performed. This means that a control sample of the cargo must be opened to perform the test. As it is not possible to open all boxes, and as smugglers hide the drug-containing boxes between regularly filled boxes, contaminated cargos can be overlooked. Investigators sometimes cannot perform the drug-test panel because they try not to arouse the smugglers' suspicion in order to follow the cargo and to find the recipient.

Aims: The objective of our studies was to define non-invasive examination techniques to investigate cargos that are suspicions to contain dissolved cocaine without leaving traces on the samples. We examined vessels containing cocaine by radiological cross-section techniques such as multidetector computed tomography (MDCT) and magnetic resonance spectroscopy (MRS).

Methods: In a previous study, we examined bottles of wine containing dissolved cocaine in different quantities using an MDCT unit. To distinguish between bottles containing red wine and those where cocaine was solved in the wine, cross sectional 2D-images have been reconstructed and the absorption of X-rays was quantified by measuring the mean density of the liquid inside the bottles. In our new study, we investigated phantoms containing cocaine dissolved in water with or without ethanol as well as cocaine dissolved in different sorts of commercially available wine by the use of a clinical magnetic resonance unit (3 tesla). To find out if dissolved cocaine could be detected, magnetic resonance spectroscopy (1H MRS) was performed.

Results: By using a MDCT-unit and measuring the mean attenuation of X-rays, it is possible to distinguish weather substances are dissolved in a liquid or not, if a comparative liquid without any solutions is available. The increase of the mean density indicates the presence of dissolved substances without the possibility to identify the substance. By using magnetic resonance spectroscopy, dissolved cocaine can be clearly identified because it produces distinctive resonances in the spectrum. In contrast to MDCT, this technique shows a high sensitivity (detection of 1 mM cocaine in wine).

Conclusions: Cross-sectional imaging techniques such as MDCT and MRS appropriated to examine cargos that are suspicious to contain dissolved cocaine. They allow to perform non-invasive investigations without leaving any trace on the cargo. While an MDCT scan can detect dissolved substances in liquids, identification of cocaine can be obtained by MR-spectroscopy.

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O33. The prevalence of drugs amongst drivers of motor vehicles in Shanghai

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Introduction: Epidemiological studies show that alcohol is an important risk factor for traffic injuries, but the increase of traffic accidents due to licit and illicit drug use has become a global and complicate public health problem.

Aim: This prospective study investigated the presence of psychoactive drugs in drivers of motor vehicles in Shanghai and its neighboring cities.

Methods: Blood samples from 10002 drivers and urine samples from 126 drivers were analysed by LC-MS-MS. Blood samples were obtained from traffic accident and traffic violation in Suzhou, Wuxi and Shanghai from 2007 to 2008. Urine samples were obtained from Health Examination Center in Shanghai Xuhui Central Hospital.

Results: Of the10002 blood samples, 10.5% tested positive for drugs (alcohol excluded). There were 15 cases (0.15%) tested positive for methamphetamine, 1 case (0.01%) for MDMA, 5 cases (0.05%) for ketamine, 46 cases (0.46%)

for benzodiazepines and 12 cases combined use of alcohol, 12 cases (0.12%) for antimanic and antidepressive drugs such as doxepin and carbamazepine. Cold medicines were the most frequently detected drugs and the frequency of cold medicine positive blood test was 8.58%. Sometimes two or more kinds of cold medicine were tested positive in the same sample. There were 478 cases (4.78%) tested positive for chlorpheniramine, 215 cases (2.15%) for pseudoephedrine, 132 cases (1.32%) for paracetamol. There were 24 cases (0.24%) tested positive for antihypertensive drug such as metoprolol and L-amlodipine, 28 cases (0.28%) for atropine, 60 cases (0.60%) for naloxone, diphenhydramine and other therapeutic drugs. Of the urine samples, 12.7 percent tested positive for drugs, without any illicit drugs detected. There were 12 cases (9.52%) tested positive for chlorpheniramine, 7 cases (5.55%) for cold medicines, including pseudoephedrine, paracetamol, methorphan.

Conclusion: In USA and European countries, the main drugs, that affect driving ability, are marijuana, benzodiazepines, barbitals, amphetamines, opiates (alcohol excluded). The presence of psychoactive drugs in blood samples from drivers of motor vehicles in Shanghai and its neighboring cities was quite different from those reports in other countries.

Keywords: prevalence, drugs, drivers of motor vehicles, Shanghai

O34. First nationwide study on driving under the influence of drugs in Switzerland

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Introduction: In January 2005, the new Swiss traffic law came into force. Switzerland, as several European countries before, introduced a "zero-tolerance" approach for driving under the influence of drugs (DUID) for some substances. According to the new law, the offender is sanctioned if THC is > 1.5 ng/ml and amphetamine, methamphetamine, MDMA, MDE, cocaine or free morphine are > 15 ng/ml in whole blood (interval of confidence ± 30%). For all other psychoactive substances, impairment must be proved in applying the so-called "three pillars expertise". At the same time the legal blood alcohol concentration (BAC) limit for driving was lowered from 0.8 to 0.5 g/kg.

Aim: The purpose of this study was to analyse the prevalence of drugs in the first year after the introduction of the revision of the Swiss Traffic Law in the population of drivers suspected of DUID.

Methods: A database was developed to collect the data from all DUID cases submitted by the Police or the Justice to the eight Swiss authorised laboratories between January and December 2005. Data collected were anonymous and included the age, gender, date and time of the event, the type of vehicle, the circumstances, the sampling time and the results of all the performed toxicological analyses. The focus was explicitly on DUID; cases of drivers who were suspected to be under the influence of ethanol only were not considered.

Results: The final study population included 4794 DUID offenders (4243 males, 543 females). The mean age of all drivers was 31 ± 12 years (range 14-92 years). One or more psychoactive drugs were detected in 89% of all analyzed blood samples. In 11% (N = 530) of the samples, neither alcohol nor drugs, were present. The most frequently encountered drugs in whole blood were cannabinoids (48% of total number of cases), ethanol (35%), cocaine (25%), opiates (10%), amphetamines (7%), benzodiazepines (6%) and methadone (5%). Other medicinal drugs such as antidepressants and benzodiazepine-like were detected less frequently. Poly-drug use was prevalent.

Conclusions: This first Swiss study points out that DUID is a serious problem on the roads in Switzerland. Further investigations will show if this situation has changed in the following years.

Keywords: driving under the influence of drugs (DUID), drugs, alcohol, Swiss legislation

O35. Mortality and causes of death among drugged drivers

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Introduction: People suspected of driving under the influence of drugs (DUID) can be studied as drugged drivers, but also as a sample of drug users. The mortality of drug users is often studied among cohorts using iv-drugs and admitted for detoxification or treatment programs. Thus, studying DUID suspects gives complementary information about the mortality of drug users.

Aims: This study analyzed mortality rates and causes of death among drugged drivers in Finland and compared them with the general Finnish population during 1993–2006.

Methods: The register data consisted of 5,832 DUID suspects apprehended by the police. A reference group of 74,809 of individuals was drawn from the general Finnish population. Deaths were traced from National Death Register. The survival and the differences in mortality hazard were estimated by using Kaplan-Meier plots and Cox regression models.

Results: Both male and female DUID suspects had almost ten times higher hazard of death in comparison with the population. Among male DUID suspects cause-specific hazards were highest when the cause of death was poisoning/overdose, violence or suicide. One fourth of DUID suspects were under the influence of drugs or alcohol at the time of death, whereas under one tenth of the population was intoxicated while dying. Male DUID suspects had twice as high hazard of death as females. Cases with a finding for one drug only were likely to survive longer than cases with poly-drug findings. DUID suspects who had a finding for medicinal drugs (especially benzodiazepines) had a higher hazard of death than DUID suspects with a finding for illicit drugs (especially amphetamines).

Conclusions: DUID suspects apprehended by the police had an increased risk for premature death, in all observed causes of death. Findings for sedatives/tranquillizers indicated excessive mortality over findings for stimulants. Preventive actions should be aimed especially at benzodiazepine users.

Keywords: Driving under influence of drugs, DUID, mortality, premature deaths

O36. Quality assurance in road traffic analyses in Switzerland

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Introduction: Swiss laboratories performing toxicological road traffic analyses have had to be authorized by the Swiss Federal Roads Office (FEDRO) for many years. In 2003 the Swiss society of legal

medicine (SSLM) was contracted by FEDRO to organize the complete quality management concerning road traffic analyses. For this purpose a multidisciplinary working group was established under the name of "road traffic commission (RTC)".

Aims: RTC has to organize external quality control, to interpret the results of these controls, to perform audits in the laboratories and to report all results to FEDRO. Furthermore the working group can be mandated for special tasks by FEDRO.

Results: As an independent organization the Quality Control Center Switzerland in Geneva manages the external quality controls in the laboratory over the past years. All tested drugs and psychoactive substances are listed in a federal instruction. The so called 'zero tolerance substances' (THC, morphine, cocaine, amphetamine, methamphetamine, MDMA and MDEA) and their metabolites have to be tested once a year, all other substances (benzodiazepines, zolpidem, phenobarbital etc.) periodically. Results over the last years show that all laboratories are generally within the confidence interval of + 30% of the mean value. In cases of nonconformities measures have to be taken immediately and reported to the working group. External audits are performed triennially but accredited laboratories can combine this audit with the approval of the Swiss Accreditation Service (SAS). During the audits a special checklist filled in by the laboratory director is assessed. Nonconformities have to be corrected.

During the process of establishing a new legislation, RTC had an opportunity of advising FEDRO.

Conclusion: In collaboration with FEDRO, RTC and hence SSLM can work actively on improving of quality assurance in road traffic toxicological analyses, and has an opportunity to bring its professional requests to the federal authorities.

Keywords: drug and driving, quality assurance, Swiss laboratories

O37. Statistical modelling of measurement errors in gas chromatographic analyses of blood alcohol content

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Introduction: Over consumption of alcohol is one of the most frequent cause of fatal accidents on the roads according to the United Nations' statistics of road traffic accidents in Europe and North America. In many countries the crime of driving under influence (DUI) has two limits of severity and the offender's penalty is derived by his/her blood alcohol content (BAC). Headspace gas chromatographic measurements of ethanol content in blood specimens from suspect drunk drivers are routinely carried out in forensics laboratories. In the widely established standard statistical framework, measurement errors in such data are represented by Gaussian distributions for the population of blood specimens at any given level of ethanol content. It is known that the variance of measurement errors increases as a function of the level of ethanol content and the standard statistical approach addresses this issue by replacing the unknown population variances by estimates derived from large samples using a linear regression model. Appropriate statistical analysis of the systematic and random components in the measurement errors is necessary in order to guarantee legally sound security corrections reported to the police authority.

Aim: Here we address this issue by developing a novel statistical approach that takes into account any potential nonlinearity in the relationship between the level of ethanol content and the variability of measurement errors.

Methods: Our method is based on standard nonparametric kernel techniques for density estimation using a large database of laboratory measurements for blood specimens. Furthermore, we address also the issue of systematic errors in the measurement process by a statistical model that incorporates the sign of the error term in the security correction calculations.

Results: Analysis of a set of spiked-in blood samples demonstrates the importance of explicitly handling the direction of the systematic errors in establishing the statistical uncertainty about the true level of ethanol content. Use of our statistical framework to aid quality control in the laboratory is also discussed.

Conclusion: Using the density estimation technique allows us to take into account the fact that the SD may not be a constant in the population of blood samples (as assumed in Jones and Shuberth, where the SD was provided by the regression model for any mean BAC). In details, our SD distribution represents the uncertainty about the SD in the population; as a consequence, the level of confidence is valid and conservative also for those individuals whose samples are subject to a higher level of variation.

References: Jones A.W. and Schuberth J. - Computer-aided headspace gas chromatography applied to Blood-Alcohol analysis: Importance of online process control - Journal of Forensic Sciences, JFSCA, Vol. 34, No.5, Sept.1989, pp. 1116-1127

Keywords: blood alcohol content, Kernel technique for density estimation, security correction

O38. Selective detection of phosphatidylethanol homologues in blood as biomarkers for alcohol misuse by LC-ESI-MS/MS

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Introduction: Phosphatidylethanol (PEth) is an abnormal phospholipid with homologous fatty acid chains (two per molecule) formed only in the presence of ethanol via the action of phospholipase D (PLD) and its use as a biomarker is currently under investigation. The mean half-life of PEth in blood from alcoholics was found to be 4 days, and PEth was still measurable after up to two weeks of sobriety. Previous methods for the analysis of PEth applied homologue-unspecific HPLC/ELSD – besides newer papers on LC-MS using ToF MS.

Aim: The purpose of this work was to i) develop an LC-MS/MS method for sensitive quantitation of PEth (16:0/16:0 and 18:1/18:1), the homologues which are currently available by a chemical supplier as reference standards, ii) to set-up a screening procedure using MRM for other homologues forms (Peth C14 to C20), and iii) apply these methods to different blood samples – from a drinking experiment and autopsy cases – for further method development (investigation of stability etc.).

Methods: Internal standard (Phosphatidyl-butanol 16:0/16:0 (Avanti Polar Lipids) was added to blood samples, sample extraction was performed using 0.4 ml 2-propanol and 0.6 ml hexane (in two steps), after evaporation samples were redissolved in 150 µl HPLC-eluents and injected into an Agilent HPLC 1100 system coupled to a Qtrap 2000 tandem-mass spectrometer (Applied Biosystems). HPLC: column: Luna C8(2), 50 × 2 mm, 3 µm; gradient elution using 2 mM ammonium acetate and methanol/acetone (95/5; v:v), MRM signals were monitored: C16:0/C16:0 M-H- m/z 675.5 – 255.2 and 437.3 and 181.0; C18:1/C18:1: M-H- 727.5 – 281.3 and 181.0 and 463.3. Internal standard: 703.5 – 255.2. The respective transitions were used for other homologues PEth forms, with respective M-H- as precursor ions.

Results: Linear calibration was performed in the range of 20 ng/mL to 1500 ng/mL for PEth 16:0/16:0 and 18:1/18:1. With this procedure detection of PEth was possible in blood samples (heart blood) from autopsy

cases (in concentration ranges of 122 to 580 ng/mL 16:0/16:0 and 405 to 1903 ng/mL for 18:1/18:1). Furthermore, Peth 18:1/16:0 or its isobaric isomer PEth 16:0/18:1 was detected. In blood from a volunteer - after a single 60 g-dose of ethanol - PEth 16:0/16:0 and 18:1/18:1 could be detected in trace level amounts below the respective LOQs. Just as the autopsy cases, Peth 18:1/16:0 was found. In this case, NaF (10 mg/mL) had been used to stabilize the analyte which seems to be labile to esterase activity.

Conclusions: By LC-MS/MS PEth homologues could be quantified specifically by LC MS/MS with the advantage compared to ELSD, where the sum of different homologue forms of PEth is detected. This opens a new field of application of PEth to uncover single or multiple heavy drinking already at a lower frequency and with a larger window of detection in blood than before.

Keywords: PEth, phosphatidylethanol, alcohol biomarker, LC-MS/MS

O39. Analysis of phosphatidylethanol in blood by non-aqueous capillary electrophoresis with on-line ESI-MS detection

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Introduction. Phosphatidylethanol (PEth) is an aberrant phospholipid formed in cell membranes in the presence of ethanol. Significant concentrations of PEth are found in the blood of chronic alcoholics. Analysis of PEth has so far been carried out with TLC, LC-ESI-MS, LC-TOF-MS or LC and evaporative light-scattering detection (ELSD). LC methods have proved to be sensitive and quite accurate, conversely requiring large amounts of organic solvents and time-consumption. Recently non-aqueous capillary electrophoresis (NACE) with UV detection has been proposed for the determination of PEth in blood.

Aims. The aim of the study was to develop a new analytical method based on NACE coupled with an online ESI-MS, thus trying to overcome the limitations related to the low selectivity of the UV detector.

Methods. Extraction of lipids was performed from blood (0.3 mL) by slowly adding under agitation of methanol (5 mL), sonication of the mixture and addition of dicholoromethane (10 mL). Once the pellets were removed by centrifugation, water (5 mL) was added to clear liquid phase. The lower organic phase was collected and gently dried under a nitrogen stream. All the experiments were performed on HP3dCE system (Agilent Technologies, CA, USA) using an uncoated fused-silica capillaries (90 cm × 100 μm i.d.). Electrophoresis was coupled to the 1100 MSD ion trap mass-spectrometer (Agilent Technologies) through a CE-ESI-MS adapter kit (Agilent Technologies). A basic solution of ammonium acetate 5 mM (pH 9) in water/methanol (80:20 v/v) was delivered as coaxial sheath liquid. The effects of variable percentages of acetonitrile (ACN), methanol (MeOH), 2-propanol, hexane, along with variable concentrations of ammonium acetate, were investigated for the separation of PEth.

Results. PEth, as all other phospholipids, is poorly soluble in aqueous media. Therefore, a non-aqueous buffer has to be used for the electrophoretic separation (NACE conditions). MeOH, ACN, and their mixtures are the most frequently employed organic solvents in NACE separations. Collectively, a separation medium composed of ACN (55% v/v), MeOH (15% v/v), isopropanol (15% v/v) and hexane (15% v/v), was finally chosen. Varying the concentration of ammonium acetate in the range of 15 and 35 mM, the best separation efficiency and signal intensity were observed at 25 mM. The suitability of the method for quantitative analysis was studied by testing selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and repeatability. The selectivity was estimated by analysing blood samples (n = 10) collected from different teetotallers (n = 5). The calibration curve obtained by plotting the ratio of the peak areas of PEth to that of IS versus concentrations, proved to be linear in the range of 0.4-15 μM. The LOD was 0,1 μM. The LOQ was found to be 0.4 μM. Within-run (intra-day) and between-run (inter-day) precision was evaluated by performing repeated injections (n = 6) of the same sample at two different concentrations (2 and 10 μM) on three different days. CV was always lower than 15%.

Conclusions. The method proved to be robust and reliable. The MS detector allowed the utilization of phosphatidylbutanol as internal standard raising both sensitivity and selectivity if compared to other previous published electrophoretic methods.

Keywords: phosphatidylethanol, PEth, electrophoresis, NACE, alcoholism

O40. Formation of oxidation products of (-)-trans-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) after exposure to oxidizing adulterants

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Introduction: Cannabis is one of the most widely used illicit drugs in Australia and in the world. The major psychoactive constituent of cannabis is (-)-trans-Δ9-tetrahydrocannabinol (THC). Cannabis use is primarily detected by identifying the presence of 11-nor-THC-9-carboxylic acid (THCCOOH), the major metabolite of THC, in biological fluids such as urine. Many oxidizing agents such as hypochlorite, hydrogen peroxide, pyridinium chlorochromate, glutaraldehyde, and nitrite have been used as urine adulterants by drug users producing a false negative test result. Although various analytical methods are available to detect the presence of some of these oxidants, there is currently no method available to detect the oxidation products of THCCOOH in urine after exposure to oxidants. Inability to detect these oxidation products may therefore invalidate many test results. In cases where specimen adulteration has occurred, currently no direct evidence of drug use by an individual can be obtained.

Aim: The objectives of this pilot study were to expose THCCOOH initially in water to two commonly available oxidizing adulterants: sodium hypochlorite and pyridinium chlorochromate, to monitor oxidation products formed in these reaction systems, and thus to provide some useful leads in future research examining the reaction of THCCOOH with these adulterants in urine. The ultimate goal of this study was to search for stable oxidation products that could be used as potential markers for monitoring urine adulteration activities among cannabis users.

Methods: THCCOOH added in water was reacted with sodium hypochlorite and pyridinium chlorochromate. Reaction products from these reactions were monitored by liquid chromatography-mass spectrometry (LC-MS). LC separation was performed on a Zorbax® XDB C8 HPLC column (150 mm × 4.6-mm i.d., 5 micron particle size). The LC eluents were monitored on a triple quadruple MS instrument operated in negative electrospray ionization mode.

Results: Three oxidation products of THCCOOH have been identified through the reaction of THCCOOH with sodium hypochlorite. Based on the MS evidence, their structures were tentatively assigned as below: one di-chlorinated and two mono-chlorinated species. A single oxidation product has been produced through the reaction of THCCOOH with pyridinium chlorochromate. Structural identification of the product is currently underway.

Conclusion: The successful detection of these novel oxidation products may allow them to be studied in urine specimens. The research findings will provide the chemical basis for development of potential markers for monitoring cannabis abuse even if the urine specimen is adulterated by hypochlorite and chlorochromate.

Keywords: THCCOOH, urine adulteration, hypochlorite, pyridinium chlorochromate

O41. Interest of qualitative and quantitative profiling of endocannabinoids for evaluation of their implication in drug addiction process

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Introduction: In the middle of the 90's, the discovery of endogenous ligands for cannabinoid receptors opened a new era in this research field. Amides and esters of arachidonic acid have been identified as these endogenous ligands. Arachidonoylethanolamide (anandamide or AEA) and 2-Arachidonoylglycerol (2-AG) seem to be the most important of these lipid messengers. In addition, virodhamine (VA), noladin ether (2-AGE), and N-arachidonoyl dopamine (NADA) have been shown to bind to CB receptors with varying affinities. During recent years, it has become more evident that the EC system is part of fundamental regulatory mechanisms in many physiological processes such as stress and anxiety responses, depression, anorexia and bulimia, schizophrenia disorders, neuroprotection, Parkinson disease, anti-proliferative effects on cancer cells, drug addiction, and atherosclerosis.

Aims: This work presents the problematic of EC analysis and the input of Information Dependant Acquisition based on hybrid triple quadrupole linear ion trap (QqQLIT) system for the profiling of these lipid mediators.

Methods: The method was developed on a LC Ultimate 3000 series (Dionex, Sunnyvale, CA, USA) coupled to a QTrap 4000 system (Applied biosystems, Concord, ON, Canada). The ECs were separated on an XTerra C18 MS column (50 × 3.0 mm i.d., 3.5 µm) with a 5 min gradient elution. For confirmatory analysis, an information-dependant acquisition experiment was performed with selected reaction monitoring (SRM) as survey scan and enhanced produced ion (EPI) as dependant scan.

Results: The assay was found to be linear in the concentration range of 0.1-5 ng/mL for AEA, 0.3-5 ng/mL for VA, 2-AGE, and NADA and 1-20 ng/mL for 2-AG using 0.5 mL of plasma. Repeatability and intermediate precision were found less than 15% over the tested concentration ranges. Under non-pathophysiological conditions, only AEA and 2-AG were actually detected in plasma with concentration ranges going from 104 to 537 pg/mL and from 2160 to 3990 pg/mL respectively. We have particularly focused our scopes on the evaluation of EC level changes in biological matrices through drug addiction and atherosclerosis processes. We will present preliminary data obtained during pilot study after administration of cannabis on human patients.

Conclusion: ECs have been shown to play a key role in regulation of many pathophysiological processes. Medical research in these different fields continues to growth in order to understand and to highlight the predominant role of EC in the CNS and peripheral tissues signalisation. The profiling of these lipids needs to develop rapid, highly sensitive and selective analytical methods.

O42. Quantitative determination of the active 'spice' ingredient JWH-018 in blood and hair by liquid chromatography - tandem mass spectrometry

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Introduction: The incense 'Spice' and similar herbal mixtures receive growing interest in the public. Consumers have reported a hallucinogenic effect from smoking 'Spice'. Recently, several synthetic cannabinoids, which have been identified as active ingredients of 'Spice', were included in the german controlled substances legislation. Possible other active ingredients remain to be identified. The legal basis of the prosecution of driving under the influence of drugs is analytical proof of psychoactive substances in the driver's blood. Drug content in hair is analyzed for example for long-term abstinence programs.

Aim: The purpose of our work was to show that JWH-018 is a relevant ingredient of 'Spice' mixtures, and to develop a fast and easily performed method for the routine quantification of JWH018 in blood and hair.

Methods: Different herbal incense mixtures were screened for their ingredients. Methanolic extractions were analysed on a GC-MS. For the detection in blood, solid phase extraction on C18-cartridges has been carried out after addition of the internal standard d7-JWH-018. Extraction of hair samples was carried out in methanol. The extracts were analysed on a LC-MS/MS-triple quadrupol system. Two transitions in 'multiple reaction monitoring' mode and the retention time were employed to provide unambigous identification of the substance.

Results: Based on the assignment of mass fragments in GC-MS spectra, several synthetic cannabinoids like JWH-018, JWH-073 and CP47,497-C8-homologue could be identified in the incense mixtures. A method for the quantitative determination of JWH-018 in blood was developed and validated. The method's limit of detection and limit of quantitation were 0.2 ng/ml and 0.6 ng/ml respectively. Other common drugs did not interfere with this highly selective quantification of JWH-018 in blood. In forensic blood samples JWH-018 concentrations were in the low to sub-nanogram per milliliter range. Results for the analysis of JWH-018 in hair in the picogram range will be presented.

Conclusion: With the presented method it is possible to detect and to quantify JWH-018 – one of the psycho-active ingredients of the incense drug 'Spice' in blood and hair.

Keywords: Spice, JWH-018, LC-MS/MS, determination in blood, determination in hair

O43. Studies on the metabolism of JWH-018 and of a homologue of CP 47,497, pharmacologically active ingredients of different misused incense ("Spice") using GC-MS and LC-MSn techniques

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Introduction: In the last few months, a new drug has conquered the cannabis market: different types of incenses (trade names "Spice", "Smoke", "Sence" and others) have widely been misused by smoking these blends of herbs. Recently, artificial endocannabinoid receptor agonists (JWH-018 and a homologue of CP 47, 497) have been found to be the pharmacologically active principles in these blends. Unfortunately, little is known about these substances.

Aim: The aim of this study was to elucidate the metabolism of JWH-018 and of a homologue of CP 47,497 (CP47).

Methods: Ethanolic extracts were prepared from incenses containing JWH-018 and CP47, respectively. After removal of the ethanol the residues were given to Wistar rats by gastric intubation and urine was collected over

24 hours. For identification, the metabolites were isolated after enzymatic or acidic cleavage of conjugates by liquid-liquid extraction (LLE) or solid-phase extraction (C18) followed by acetylation and GC-MS analysis. For LC-MS the underivatized extracts were used. Metabolites were identified by interpretation of the EI mass spectra (GC-MS) and enhanced product ion (EPI) scan mass spectra (LC-MS). MS3 experiments allowed differentiation of isomeric metabolites.

Results: The parent compounds JWH-018 and CP47 could be found in the urine extracts. For JWH-018, the N-dealkyl metabolite could be detected as well as the hydroxylated metabolite. The highest signals could be observed for the hydroxylated N-dealkyl metabolites. Hydroxylation took place in the side chain and in both aromatic systems, the naphthalene and the indol part, which could be shown by mass shift of the corresponding fragments and by MS3 experiments. For CP47, several hydroxylated metabolites could be identified. Aliphatic hydroxyl groups could be differentiated from aromatic hydroxyl groups by different fragmentation patterns (loss of water/acetic acid for aliphatic hydroxyl groups).

Conclusion: JWH-018 and CP 47 are extensively metabolized in rats. According to our experience similar metabolic patterns can be expected in humans. Therefore, screening procedures for these drugs in urine should include not only the parent compound but also the corresponding metabolites.

Keywords: JWH-018, Cp 47,497, metabolism, GC-MS, LC-MSn

O44. Cannabis intoxication with perceptual disturbances under clinical setting

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Introduction: Short-term psychiatric effects have been described for cannabis, but rarely under clinical setting.

Aims: We report three cases of healthy male young subjects who were occasional cannabis users without known psychiatric history who developed anxiety attacks, panic crisis, and transient perceptual disturbances (visual hallucinations, depersonalization, paranoid feelings and derealization) following oral or smoking administration of cannabis. In contrast to most other case reports where subjects characteristics and history of drug use, circumstances, doses and blood concentrations are unknown, the three cases reported here happened under experimental conditions.

Methods: Among the three cases, two happened among a series of 8 healthy male volunteers included in a study testing the psychomotor effects of oral cannabis (one case received 20 mg dronabinol and the other a decoction of 16.6 mg THC). The third critical case happened in a series of 16 subjects involved in a study to assess the effects of cannabis smoking on a tracking task carried out during a functional magnetic resonance imaging (fMRI) experiment. The two studies were approved by the ethical commissions.

Results: no opiates, amphetamines, cocaine, and benzodiazepines were found in urine and no breath alcohol was detected before each session. The ingested (16.6 mg THC or 20 mg dronabinol) or inhaled dose (joint=0.8 g, 11% THC, 10 puffs of 2 s, no tobacco added), the time-events of effects on behavior, willingness to drive, and performance as well as the cannabinoid whole blood levels were documented. While the oral route of administration achieved only limited blood concentrations (less than 4.7 ng/mL (dronabinol) and 3.9 ng/mL (THC)), significant psychotic reactions occurred. In contrast, following inhalation, much larger THC blood levels (peak concentration: 143 ng/mL) were found. The psychotic symptoms started at the end of the inhalation phase and continued during the distribution phase when the THC levels rapidly decreased from 143 to 17 ng/mL in 16 min. Two tablets of Temesta 1 mg (lorazepam) were then successively administered to this volunteer to ease anxiety and hallucinations. In this latter case, MRI and fMRI brain imaging performed before cannabis smoking did not disclose any obvious anatomical or brain functioning anomalies. All 3 cases were withdrawn from the studies because of these unwanted side effects.

Conclusions: The THC and dronabinol maximal blood levels were found to be poor predictors of possible psychiatric side-effects. Furthermore, these unwanted symptoms seem to be more frequent than expected, despite the relative medium dose of cannabinoids administered and the careful selection of the subjects.

Keywords: cannabis, clinical study, psychotic symptoms

O45. Metabolism of the new designer drug mephedrone and toxicological detection of the beta keto designer drugs mephedrone, butylone and methylone in urine

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Introduction: Beta keto (bk) designer drugs are a new class of drugs of abuse. In contrast to mephedrone (2-methylamino-1-p-tolylpropane-1-one), the metabolism of butylone (2-methylamino-1-(3,4-methylenedioxyphenyl) butan-1-one, bk-MBDB) and methylone (3,4-methylenedioxymethcathinone, bk-MDMA) has already been investigated. So far, these designer drugs have not yet been included in our systematic toxicological analysis (STA).

Aim: The first aim of the presented work was to study the metabolism of mephedrone and to incorporate all of the above-mentioned bk-designer drugs into our STA. The second aim was to check for suitability of our rat model by comparing incurred rat urine samples with human urine samples from mephedrone and butylone users.

Methods: For the metabolism study, urine samples from male Wistar rats (20 mg/kg BW) were extracted (liquid-liquid or Isolute Confirm HCX cartridges) after enzymatic cleavage of conjugates. After extraction and acetylation, the metabolites were separated and identified by GC–MS in the electron ionisation and in the positive chemical ionisation mode. For toxicological detection, a common users dose corresponding to 1 mg/kg BW were administered to rats and urine was collected over a 24 h period. Human urine submitted to our laboratory for toxicological analysis was collected approximately 6 hours after intake of an unknown amount of butylone and mephedrone. The rat and human urine samples were analyzed using our STA based on an acid hydrolysis followed by liquid-liquid extraction, acetylation and analysis via full-scan GC-MS. Finally, the results from the metabolism and screening studies in rats were compared to those obtained from the patients' urine to verify the suitability of the used rat model.

Results: Analysis of the rat and human samples revealed the following main metabolic steps for mephedrone: N-demethylation to the primary amine, reduction of the keto moiety to the respective alcohol and oxidation of the tolyl moiety to the corresponding alcohols and carboxylic acids. The metabolites of butylone and mephedrone detected in rat urine could also be found in human urine samples. Using our STA, the parent compounds and N-demethyl metabolites could be detected in rat urine after a common user's dose as well as in the patients' urine samples in the case of mephedrone and butylone.

Conclusion: Besides the elucidation of the metabolism of the new designer drug mephedrone, we were able to show, that our STA was suitable to proof

an intake of at least butylone and/or mephedrone in human urine. These examples showed again that the used rat model was suitable to predict the qualitative metabolism and detectability of drugs in human urine.

Keywords: designer drugs, butylone, mephedrone, methylone, metabolism, STA

O46. Studies on the metabolism of the Kratom alkaloid paynantheine in rat and human urine using liquid chromatography-linear ion trap mass spectrometry

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Introduction: Besides more than twenty alkaloids, mitragynine (MG, 70%) and paynantheine (PAY, 10%) are the two most abundant alkaloids of the Thai medicinal plant *Mitragyna speciosa* (Kratom), which is used as herbal drug of abuse. Monitoring of Kratom intake in urine should cover both main alkaloids and/or their metabolites. Studies on mitragynine have already been described.

Aim: The aim of the presented study was to investigate the phase I and II metabolism of PAY in rat and human urine using liquid chromatography-linear ion trap mass spectrometry. These data should be the basis for establishing an additional marker for Kratom use.

Methods: Urine samples (1 ml) from male Wistar rats, which had been administered a 40 mg/kg BW dose of paynantheine, were extracted after enzymatic conjugate cleavage by SPE (HCX) or directly by SPE (C18). The same procedure was conducted with several human urine samples of different Kratom users submitted to the authors' laboratory. The metabolites were identified by the Thermo Fisher Linear Ion Trap LXQ (details in Philipp *et al.*, 2009) in the full-scan and data dependant MSn mode.

Results: Besides PAY, the following phase I metabolites could be identified: 9-O-demethyl PAY, 16-carboxy PAY, 9-O-demethyl-16-carboxy PAY, 17-O-demethyl-16,17-dihydro PAY, 9,17-O-bisdemethyl-16,17-dihydro PAY, 17-carboxy-16,17-dihydro PAY, and 9-O-demethyl-17-carboxy-16,17-dihydro PAY. The identified metabolites indicated that PAY was metabolized by the same steps as mitragynine, *i.e.* by hydrolysis of the methylester in position 16, O-demethylation of the 9-methoxy group and of the 17-methoxy group, followed, via the intermediate aldehydes, by oxidation to carboxylic acids or reduction to alcohols and combinations of some steps. In rats and humans, several metabolites were excreted as glucuronides. In rat urine, the sulfate of 9,17-O-bisdemethyl-16,17-dihydro PAY could be detected, but in human urine that of 9-O-demethyl PAY.

Conclusion: The metabolism studies showed that PAY was extensively metabolized in rats and humans with some differences, particularly in phase II metabolism. As additional markers for a Kratom abuse, PAY and several metabolites could be identified in human urine besides MG and its metabolites.

Keywords: Kratom abuse, mitragynine; paynantheine; metabolism, LC-MS

O47. Analysis of sulfate conjugates improves detection of some anabolic steroids in urine

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Introduction: Anabolic-androgenic steroids (AAS) are abused to enhance physical performance. AAS comprise the greatest number of positive findings in sports doping control. Laboratories constantly develop analytical procedures for more efficient retrospective detection of anabolic drugs. Existing methods employ GC-MS and LC-MS technology for screening and confirmation of metabolites in urinary glucuronide steroid fraction. During the last few years considerable advances in analytical instrumentation have made possible the development of more sensitive and selective tests for steroid metabolites to allow detection for longer periods of time after drug use.

Aim: The purpose of this work is to further prolong the detection window of AAS by use of 'minor' long-term metabolites in urinary sulfate fraction, which is currently discarded in existing routine procedures.

Methods: The glucuronide and sulfate conjugated steroid fractions are separated by consecutive extractions of 2 mL of urine on C18 solid phase cartridges and intermediate enzymatic hydrolysis, first with glucuronidase from *Eischerischia coli* enzyme and then with an Abalone preparation containing arylsulfatase. Cleaved steroids are derivatized with N-methyl-N-trimethyl-silyltrifluoroacetamide (MSTFA)/ NH4I/ Ethanthiol (1000v:2w:3v) reagent. Fast GC/MS analysis is performed on both single- and triple-quadrupole instruments in EI mode.

Results: A striking difference between composition of metabolites in glucuronide and sulfate conjugated fractions is observed for anabolic steroids mesterolone, methenolone and drostanolone. A major glucuronide conjugated metabolite, 3α-hydroxy-3-one, of these drugs is commonly targeted for detection. None of these are found after enzymatic hydrolysis with arylsulfatase. Instead, sulfate fraction contains two prominent metabolites: 3β-hydroxy isomer and hydroxylated 3,17-dione metabolite, which favor sulfate conjugation. Both sulfate conjugated metabolites represent a minor fraction in total excretion, but they remain longer in the system and can be detected after the major 3α-hydroxy metabolite is gone. A single therapeutic dose of methenolone can be reliably detected for about four days by monitoring a major metabolite and a parent drug excreted in urine as glucuronides. This time window doubles by monitoring a specific sulfate conjugated metabolite, tentatively identified as 16β-hydroxy-1-methyl-5a-androst-1-en-3,17-dione. Mass spectrometric detection of this metabolite performed in the selected reaction monitoring on a triple quadrupole instrument further increases the detection window to 14 days.

Conclusion: Analysis of sulfate conjugates improves reterospective detection for some anabolic steroids.

Keywords: Anabolic steroid metabolites, sulfate conjugated, long-term detection

O48. Determination of testosterone misuse in sport, an international comparative study

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Introduction: Urinary steroid profiling is used in doping controls to detect testosterone abuse. A testosterone over epitestosterone (T/E) ratio exceeding 4.0 is considered as suspicious of testosterone administration, irrespectively of individual heterogeneous factors such as the athlete's ethnicity. A deletion polymorphism in the UGT2B17 gene was demonstrated to account for a significant part of the inter-individual variability in the T/E between Caucasians and Asians. However, the anti-doping strategy includes the determination of carbon isotope ratio on androgen metabolites which has been demonstrated to be reliable for the direct detection of testosterone misuse. Herein, we examined the profiles and the variability in the 13C/12C-ratios of urinary steroids in a widely heterogeneous cohort of professional soccer players residing in different world countries (Argentina, Italy, Japan, South-Africa, Switzerland and Uganda).

Aim: The determination of threshold values based on genotype information and diet specific of the ethnicity is expected to enhance significantly the detection of testosterone misuse.
Methods: The steroid profile of 57 Africans, 32 Asians, 50 Caucasians and 32 Hispanics was determined by gas chromatography-mass spectrometry. The carbon isotope ratio of selected androgens in urine specimens were determined by means of gas chromatography/combustion/isotope ratio mass spectrometry (GC-C-IRMS).
Results: Significant differences have been observed between all ethnic groups. After estimation of the prevalence of the UGT2B17 deletion/deletion genotype (African:22%; Asian:81%; Caucasian:10%; Hispanic:7%), ethnic-specific thresholds were developed for a specificity of 99% for the T/E (African:5.6; Asian:3.8; Caucasian:5.7; Hispanic:5.8). Italian and Swiss populations recorded an enrichment in 13C of the urinary steroids with respect to the other groups, thereby supporting consumption of a relatively larger proportion of C3 plants in their diet. Noteworthy, detection criteria based on the difference in the carbon isotope ratio of androsterone and pregnanediol for each population were well below the established threshold value for positive cases.
Conclusion: These profiling results demonstrate that a unique and nonspecific threshold to evidence testosterone misuse is not fit for purpose. In addition, the carbon isotopic ratio from these different diet groups highlight the importance to adapt the criteria for increasing the sensitivity in the detection of exogenous testosterone. In conclusion, it may be emphasized that combining the use of isotope ratio mass spectrometry including refined interpretation criteria for positivity and the subject-based profiling of steroids will most probably improve the efficiency of the confirmatory test.
Keywords: steroids profiling, IRMS, doping

O49. Doping control analysis of recombinant human erythropoietin and darbepoetin alfa in equine plasma by nano liquid chromatography-tandem mass spectrometry

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Introduction: Recombinant human erythropoietin (rhEPO) and darbepoetin alfa (DPO) are synthetic analogues of the endogenous hormone erythropoietin (EPO). These erythropoiesis-stimulating agents have the ability to stimulate the production of red blood cells and are commercially available for the treatment of anemia in humans. These drugs are believed to have performance enhancing effects due to their stimulation of red blood cell production, thereby improving delivery of oxygen to the muscle tissues. As such, they are prohibited by authorities in both human and equine sports. The method officially adopted by the World Anti Doping Agency (WADA) for the confirmation of rhEPO and/or DPO (rhEPO/DPO) in human blood is based on electrophoresis in combination with Western blotting. A major drawback of the WADA method is the lack of definitive mass spectral data for the confirmation of a positive finding. Recently, a liquid chromatography-tandem mass spectrometry (LC/MS/MS) method for the detection and confirmation of rhEPO and DPO in equine plasma was reported by F. Guan *et al.* [1]. However, we have encountered difficulties reproducing their results at the reported sensitivity. This paper presented an alternative method for the detection and confirmation of rhEPO/DPO in equine plasma. The procedures involved immunoaffinity extraction using Dynabeads followed by trypsin digestion. Detection and confirmation were achieved by monitoring a unique peptide of rhEPO/DPO using nano liquid chromatography-tandem mass spectrometry equipped with a nanospray ESI source.
Aim: To develop a sensitive and reliable nano LC-SRM method for the confirmation of rhEPO/DPO in equine plasma.
Method: The rhEPO/DPO in plasma samples were extracted using magnetic beads (Dynabeads) coated with rhEPO antibody. The captured proteins were eluted off the magnetic beads using only 250 µL of elution buffer, followed by trypsin digestion at 37 °C for 3 hours. The LC used was a multi-dimensional nano liquid chromatography system equipped with a nano LC autosampler. The mixture of the digested sample (100 µL) was first injected to the trap column for concentration. Chromatographic separation of the peptide 46VNFYAWK52 (the T6 peptide), unique for rhEPO and DPO, was achieved on a ZC-15-C18 SBWX-150 analytical column with gradient elution at a flow rate of 1 µL/min. Confirmation of the targeted peptide was performed by monitoring four specific SRM transitions using an API 4000 QTrap mass spectrometer operating in the positive nanospray ionisation mode.
Results: Plasma samples fortified with rhEPO at sub ppb levels could be consistently detected, with limit of confirmation at 0.2 ng/mL. No significant matrix interference was observed in blank plasma samples at the retention time of the target transitions. Both retention times and relative abundances of the product ions from spiked samples matched well with those from the T6 peptide standard, easily meeting all criteria set forth in "Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry" published by the Association of Official Racing Chemists (AORC).
Conclusions: A simplified and reliable LC/SRM method has been developed for the confirmation of a unique peptide fragment of rhEPO/DPO in equine plasma. The method provides an effective tool to monitor the abuse of rhEPO/DPO in horses.
Reference: [1] Guan, F.; Uboh, C.; Soma, L.; Birks, E.; Chan, J.; Mitchell, J.; You, Y.; Rudy, J.; Xu, F.; Li, X.; Mbuy, G.; Anal. Chem. 2007; 79: 4627-4635.
Keywords: recombinant human erythropoietin, darbepoetin alfa, equine, nanospray, liquid chromatography-mass spectrometry

O50. Fast screening and confirmation of doping agents by UHPLC-QTOF-MS/MS

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Introduction: The general strategy to perform anti-doping analysis starts with a screening followed by a confirmatory step when a sample is suspected to be positive. The screening step should be fast, generic and able to highlight any sample that may contain a prohibited substance by avoiding false negative and reducing false positive results. The confirmatory step is a dedicated procedure comprising a selective sample preparation and detection mode.
Aim: The purpose of the study is to develop rapid screening and selective confirmatory strategies to detect and identify 103 doping agents in urine.
Methods: For the screening, urine samples were simply diluted by a factor 2 with ultra-pure water and directly injected ("dilute and shoot") in the ultra-high-pressure liquid chromatography (UHPLC). The UHPLC separation was performed in two gradients (ESI positive and negative) from 5/95 to 95/5% of MeCN/Water containing 0.1% formic acid. The gradient analysis time is 9 min including 3 min reequilibration. Analytes detection was performed in full scan mode on a quadrupole time-of-flight (QTOF) mass spectrometer by acquiring the exact mass of the protonated (ESI positive) or deprotonated (ESI negative) molecular ion. For the confirmatory analysis, urine samples were extracted on SPE 96-well plate with mixed-mode cation (MCX) for basic and neutral compounds or anion exchange (MAX) sorbents for acidic molecules. The analytes were eluted in 3 min (including 1.5 min reequilibration) with a

gradient from 5/95 to 95/5% of MeCN/Water containing 0.1% formic acid. Analytes confirmation was performed in MS and MS/MS mode on a QTOF mass spectrometer.

Results: In the screening and confirmatory analysis, basic and neutral analytes were analysed in the positive ESI mode, whereas acidic compounds were analysed in the negative mode. The analyte identification was based on retention time (tR) and exact mass measurement. "Dilute and shoot" was used as a generic sample treatment in the screening procedure, but matrix effect (*e.g.*, ion suppression) cannot be avoided. However, the sensitivity was sufficient for all analytes to reach the minimal required performance limit (MRPL) required by the World Anti Doping Agency (WADA). To avoid time-consuming confirmatory analysis of false positive samples, a pre-confirmatory step was added. It consists of the sample re-injection, the acquisition of MS/MS spectra and the comparison to reference material. For the confirmatory analysis, urine samples were extracted by SPE allowing a pre-concentration of the analyte. A fast chromatographic separation was developed as a single analyte has to be confirmed. A dedicated QTOF-MS and MS/MS acquisition was performed to acquire within the same run a parallel scanning of two functions. Low collision energy was applied in the first channel to obtain the protonated molecular ion (QTOF-MS), while dedicated collision energy was set in the second channel to obtain fragmented ions (QTOF-MS/MS). Enough identification points were obtained to compare the spectra with reference material and negative urine sample. Finally, the entire process was validated and matrix effects quantified.

Conclusion: Thanks to the coupling of UHPLC with the QTOF mass spectrometer, high tR repeatability, sensitivity, mass accuracy and mass resolution over a broad mass range were obtained. The method was sensitive, robust and reliable enough to detect and identify doping agents in urine.

Keywords: screening, confirmatory analysis, UHPLC, QTOF, doping agents

O51. Smoked cannabis and doping control: looking for the wrong target analyte?

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Introduction: Since 2004, cannabis is prohibited by the World Anti-Doping Agency (WADA) for all sports in competition. In the years since then, about half of all positive doping cases in Switzerland have been related to cannabis consumption. In most cases, the athletes plausibly claim to have consumed cannabis several days or even weeks before competition and only for recreational purposes not related to competition. In doping analysis, the target analyte in urine samples is 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), the reporting threshold for laboratories is 15 ng/mL. However, the wide detection window of this long-term THC metabolite in urine does not allow a conclusion concerning the time of consumption or the impact on the physical performance.

Aim: The purpose of the present pharmacokinetic study on volunteers was to evaluate target analytes with shorter urinary excretion time. Subsequently, urines from athletes tested positive for cannabis should be reanalyzed including these analytes.

Methods: In an one-session clinical trial (approved by IRB, Swissmedic, and Federal Office of Public Health), 12 healthy, male volunteers (age 26 ± 3 yrs, BMI 24 ± 2 kg/m2) with cannabis experience (> once/month) smoked a Cannabis cigarette standardized to 70 mg THC/cigarette (Bedrobinol® 7%, Dutch Office for Medicinal Cannabis) following a paced-puffing procedure. Plasma and urine was collected up to 8 h and 11 days, respectively. Total THC, 11-hydroxy-THC (THC-OH), and THC-COOH were determined after enzymatic hydrolyzation followed by SPE and GC/MS-SIM. The limit of quantitation (LOQ) for all analytes was 0.1 ng/mL. Visual analog scales (VAS) and vital functions were used for monitoring psychological and somatic side-effects at every timepoint of specimen collection (up to 480 min).

Results: Eight puffs delivered a mean THC dose of 45 mg. Mean plasma levels of total THC, THC-OH and THC-COOH were measured in the range of 0.1-20.9, 0.1-1.8, and 1.8-7.5 ng/mL, respectively. Peak concentrations were observed at 5, 10, and 90 min. Mean urine levels were measured in the range of 0.1-0.7, 0.10-6.2, and 0.1-13.4 ng/mL, respectively. The detection windows were 2-8, 2-96, and 2-120 h. No or only mild effects were observed, such as dry mouth, sedation, and tachycardia. Besides high to very high THC-COOH levels (0-978 ng/mL), THC (0.1-24 ng/mL) and THC-OH (1-234 ng/mL) were found in 90 and 96% of the cannabis-positive urines from athletes.

Conclusion: Instead of or in addition to THC-COOH, the pharmacologically active THC and THC-OH should be the target analytes for doping urine analysis. This would allow the estimation of more recent Cannabis consumption, probably influencing performance during competition.

Keywords: cannabis, doping, clinical trial, plasma and urine levels, athlete's samples

O52. Ethyl glucuronide in hair: sensitivity, specificity, and factors potentially influencing its performance

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Introduction: In recent years ethyl glucuronide in hair (HEtG) has been gaining increasing interest as a biomarker of chronic alcohol drinking. Studies conducted so far have shown that HEtG provides high sensitivity and specificity, although their conclusions might have been affected by the use of non fully validated analytical methods for HEtG determination and/or the small number of cases examined.

Aims: This study aimed at the definition of a cut-off level for ethyl glucuronide in hair (HEtG) able to effectively discriminate a heavy drinking behaviour. Moreover, the influence of factors known to affect ethanol metabolism and/or the diagnostic power of other markers of ethanol use, as well as of issues possibly affecting substance incorporation into hair (*e.g.* hair natural colour, cosmetic treatments and hygienic habits) was evaluated.

Methods: HEtG was determined in the 3 cm proximal segment of 98 volunteers among teetotallers, social drinkers, and heavy drinkers, by a fully validated LC-MS-MS method providing adequate selectivity and sensitivity of detection (LLOQ of 3 pg/mg). A written anonymous questionnaire was administered to participants in order to estimate their Ethanol Daily Intake (EDI) as well as to collect data on factors possibly affecting HEtG.

Results: The HEtG cut-off level providing the best sensitivity and specificity at detecting heavy drinking was 27 pg/mg. Sensitivity and specificity of HEtG at detecting a 3-month EDI of 60 g/day or higher were 0.92 and 0.96, respectively. None of the factors examined, including age, gender, body mass index, tobacco smoke, prevalence in the use of wine or beer, hair colour, cosmetic treatments or hygienic habits was found to significantly influence marker performance. However, the slight differences in HEtG performance observed for some factors (*e.g.* body mass index, smoke, and hair treatments) require further studies on larger groups of individuals in order to assess more precisely their influence.

Conclusions: Our results further confirm that HEtG is a sensitive and specific marker of chronic heavy drinking.

Keywords: alcohol, alcoholism, hair ethyl glucuronide, sensitivity and specificity

O53. Hair analysis for anabolic steroids: the time courses in guinea pigs

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Introduction: Hair analysis is gaining increasing attention for its unique potential in the control of doping. But relatively few publications are available in the field of doping analysis based on hair samples.

Aims: the goals of the present study were to study the time courses of the concentrations of 8 anabolic steroids in guinea pig hair and to investigate the metabolites of anabolic steroids in hair.

Methods: Sensitive, specific, and reproducible methods for the quantitative determination of 8 anabolic steroids in hair have been developed using LC/MS/MS and GC/MS/MS. After shaving black hair, methyltestosterone, stanozolol, methandienone, nandrolone, trenbolone, boldenone, methenolone and DHEA were administered intraperitoneally in guinea pigs. After the first injection, black segments were collected.

Results: The analysis of hair segments revealed the distribution of anabolic steroids in guinea pig hair. Of the metabolites, only 6Δhydroxymetandienone was detected. The time courses of the concentrations of the steroids except methenolone in hair demonstrated that the peak concentrations of all the targets were reached on days 2-4 except for stanozolol, which peaked on day 10 after administration.

Conclusions: The major components in hair are the parent anabolic steroids. The concentrations in hair appeared to be related to the physicochemical properties and to the dosage. Such studies also provide basic data that will be useful in the application of hair analysis in the control of doping and in the interpretation of results.

Keywords: anabolic steroids, hair analysis, LC/MS/MS, GC/MS/MS, time course, doping

O54. Hair analysis for the most common drugs of abuse in young children

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Introduction: In the last couple of years the demand for hair testing in young children has increased considerably. Welfare agencies, family courts and child protection units are requesting hair testing as a supplementary analysis to blood and urine, due to a larger window of opportunity in detecting drugs of abuse. Four hair samples from four separate cases are presented following LC/MS/MS determination of drugs of abuse with subsequent interpretation.

Methods: Hair specimens were collected from 4 young children for the determination of drugs. Of these four children, three presented ill to hospital emergency departments with the possibility of being under the influence of unknown substances. The remaining child was ordered undergo hair analysis by the legal courts as a result of custody issues with drug using parents. Of the children which presented in hospital, simple immunoassay screens revealed the presence of illicit substances (including amphetamines and opioids). Where possible and upon direction of the relevant welfare agencies or police investigators segmental analysis was conducted to determine the alleged administration of illicit drugs during certain periods of the young child's life. Common drugs of abuse were confirmed using LC/MS/MS (Agilent 1100 LC coupled with Applied Biosystems 3200Q trap) as per method described previously (Staikos *et al.*, 2009 TIAFT).

Results: Methamphetamine was confirmed in all of the 4 children's hair, ranging from 0.02 - 10 ng/mg. Amphetamine, the metabolite of methamphetamine or a drug in its own right, was confirmed in only 3 of the children, ranging from 0.02 - 2 ng/mg. Additional drugs such as methadone and its metabolite EDDP along with other opioids such as morphine, codeine and oxycodone were also confirmed in hair. Various benzodiazepines such as diazepam, oxazepam, alprazolam, clonazepam, and the metabolite 7-amino-clonazepam along with tramadol and ketamine, were also confirmed.

Conclusions: Drug testing in hair enables the detection of drugs when urine or blood are no longer available. This type of analysis is particularly useful for children who are often the innocent victims of parents who either administer drugs to their children or use drugs in their presence often leading to adverse consequences.

References: Staikos V., Beyer J., Gerostamoulos D., Drummer OH.. A targeted screening method for the most common drugs of abuse in hair using LCMS/MS, TIAFT 2009 - 47th TIAFT Annual Meeting, Geneva.

O55. Qualitative identification of ten benzodiazepines and ketamine in hair samples with application to drug-facilitated sexual assault investigations

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Introduction: Victims of alleged drug-facilitated sexual assault (DFSA) often come forward long after traditional toxicological specimens such as blood and urine are useful to test. Hair testing offers the advantage of a much longer detection window than blood and urine. Conclusive identification of a drug commonly associated with DFSA in the hair growth corresponding to the incident under investigation in a victim who has reported symptoms consistent with being drugged may help aid in the identification of a DFSA case.

Aims: The aim of this method was to develop and validate a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method capable of conclusively identifying eleven different drugs commonly associated with DFSA at picogram per milligram levels in hair. The identification of each analyte was based on the retention time and the comparison of four MS/MS transitions.

Methods: Hair samples were segmented into 1-2 cm segments, depending on the thickness of the hair sample. Samples were cryoground under liquid nitrogen resulting in a fine hair powder. Fifty milligrams of hair powder were extracted overnight in methanol after the addition of d5-diazepam and d7-flunitrazepam (10 pg/mg) as internal standards. Methanol extracts were concentrated, reconstituted in buffer, and extracted by solid phase extraction. Final extracts were analyzed by LC/MS/MS in the positive ion electrospray mode using an ABI400 QTRAP coupled to a Symbiosis operated in the LC mode. Four transitions were monitored for each analyte, to ensure specificity. Additionally, ten hair samples from different donors were tested to verify that endogenous compounds do not interfere with the assay. Analytes targeted were alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, midazolam, nordiazepam, oxazepam, temazepam, triazolam and ketamine.

Results: Limits of detection for each analyte were determined by extracting samples spiked with known amounts of drug at decreasing concentrations in triplicate. The LOD for each analyte was taken as the concentration at which each MS/MS transition for a given analyte gave a signal to noise ratio using ABI's Analyst software above ten. Detection limits ranged from 2-5 pg/mg for each analyte. Average ion suppression for each analyte ranged from 5% ion enhancement to 36% ion suppression.

This method has been successfully applied to dozens of samples in suspected DFSA cases. Positive findings for clonazepam, diazepam and nordiazepam will be presented.

Conclusions: Four MS/MS transitions coupled to retention time have been used to screen hair samples for pg/mg levels of ten benzodiazepines and ketamine. This method has proven to be applicable to DFSA investigations when traditional toxicology specimens such as blood and urine were not collected soon enough after the incident under investigation.

Keywords: hair testing, DFSA, LC/MS/MS

O56. Interpretation of GHB concentrations in hair. Application in drug-facilited crime and abuse cases

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Introduction: Gamma-hydroxybutyric acid, or GHB, is a substance naturally present within mammal species as neurotransmitter or neuromodulator. It is therapeutically used as an anesthetic, for treatment of insomnia, for alcohol and opiates withdrawal syndrome, and in cerebrovascular disorders. GHB can be misused by ravers or club attendees for recreational purposes (euphoria, reduced inhibition, etc.), but it is also involved in criminal offenses (drug-facilited crimes). Due to the fact that GHB detection window is very short in blood and urine, the potential use of hair to document GHB exposure has been increasely investigated since many years.

Methods: Hair strands are twice decontaminated in methylene for 2 minutes, dried and segmented (3 mm-long segments in case of single exposure investigation and 1 cm to full length in case of chronic abuse). After a thin grinding of the specimen and weighting (5 to 10 mg for 3 mm-long segments and 20-30 mg for longer segments), the specimen is incubated in 0.5 ml 0.01 N NaOH, 16h at 56 °C in presence of GHB-d6 (10 ng) used as internal standard. After cooling, the homogenate is neutralized and extracted with ethylacetate in acidic conditions. The organic phase is evaporated to dryness before silylation. Analyses are performed on a Agilent 6890 gas chromatograph coupled to a MicroMass QuatroMicro GC operated in electronic impact mode of ionization at 70 eV. Detection is achieved in MRM mode (MS/MS) after induced collision with argon and offset voltage.

Results and discussion: Physiological GHB concentrations reported in the literature are in the range 0.5 to 12 ng/mg with no influence due to sex and hair color. No variation of concentrations is observed along the hair shaft in controlled subjects, except for the proximal segment, due to a major incorporation through sweat. This demonstrates that endogenous levels, for each single subjects, are constant during hair growth. In cases of chronic abuse of GHB (n=13), the observed concentrations ranges from 9.2 to 229.1 ng/mg. In drug-facilited crimes, the solution is to use each subject as his own control with an increase of GHB concentration in the segment corresponding to the period of the offense. In drug-facilited crimes, the increase of GHB concentration along the hair shat ranges generally from 1.5 to 4.5 times the physiological concentration of the subject.

O57. Use of Rivotril® in drug-facilitated sexual assault: victim and/or user?

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Aims: We report here a case of a young woman expose to clonazepam (Rivotril®). Hair sample analysis done on Day 3 specimen showed presence of 7-aminoclonazepam (clonazepam metabolite). This would suggest that this young woman was not trustful. At the end, with the complete analytical investigation panel it was possible to get to a conclusion.

Forensic Case: A 23 years old woman wake up in her house without any short term remembers, with a bloody face and confused. The day before she was with a man in his car. She remembers drinking an orange juice with whitish sediment floating in it. 3 days after, she filled a claim. Blood and hair specimens are sampled in a hospital. Her clothes and underwear are kept and analysed. 6 months after, hair specimens are samples from the presumed victim and assailant.

Methodes: Blood samples were analysed using immunoassays tests, liquid chromatography couple to a quad tandem mass spectrometer (LC/TMS) and gas chromatography couple to a quad ion trappe (GC/ITMS). For all chromatographic methods 2 liquid / liquid extraction (LLE) in acidic and basic media with chloro-1-Butan were done. Drugs identification is done using GC/ITMS and LC/TMS. Day 3 brown hair sample strand analysis of the presumed victim was done on mixed up (none oriented from root to tip) specimen. It was decontaminated and cut into 1 mm segment. It was then incubated in sodium hydroxide 0.1 N at 95 °C for 15 minutes. 7-aminoflunitrazepam was used as internal standard [1]. Month 6 brown hair sample strand analysis was done using similar protocol. Semen search was done on the underwear after humidification with optical microscope using Florence revelation test. Spots founds on the victim trousers were analysed after being cutting off using the same protocol applied for blood samples.

Results: Semen search was negative. Trousers spot were made of clonazepam. This was confirmed with the victim blood analysis in witch 7-aminoclonazepam was found at 37 ng/mL. Day 3 hair sample analysis gave a concentration at 105 pg/mg for 7-aminoclonazepam. Month 6 young woman's hair specimen analysis gave the following results for 7-aminoclonazepam: 13 pg/mL (0-4 cm), 28 pg/mg (4-8 cm) and 15 pg/mg (8-12 cm). For the presumed assailant concentrations were 1 pg/mg (0-2 cm), 8 pg/mg (2-4 cm), 46 pg/mg (4-6 cm) and 90 pg/mg (6-8 cm).

Conclusion: Despite anamnesis, victim's medical file and the presumed assailant's track records showing that it was a clonazepam user, interpretation of the hair sample analysis was tricky. 7-aminoclonazépam concentration in blood at Day 3 and the identification of clonazepam in trousers spots lead to the conclusion that the victim was exposed to Rivotril® when the aggression occurred. At the end the recidivist assailant was convicted by 4 years jail with compulsory medical follow up and was register on the national sexual delinquent database.

References: 1. Chèze M. et coll. Hair analysis by liquid chromatography-tandem mass spectrometry in toxological investigation drug-facilitated crimes: report of 128 cases over the period june 2003–may 2004 in metropolitan Paris. Forensic Sci. Int. 2005; 153(1): 3-10.

Keywords: drug-facilitated sexual assault, Rivotril®, clonazeapam, hair sample

O58. Validation of a MALDI-based screening method for the detection of cocaine in hair

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Introduction. Considering the increasing abuse of cocaine, currently one of the most widespread abused drugs in the world, it has become important to adopt simple and fast screening methods to detect cocaine in a large number of hair samples. Matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS), due to inherent characteristics such as the soft ionisation, the privileged production of molecular species, the better tolerance with respect to electrospray ionisation (ESI)-MS to interference from salts and buffers, and the simplicity of sample preparation, is ideally suited for simultaneous, rapid and high-throughput analyses of complex mixtures and might be employed as a screening method for cocaine detection in hair.

Aims. The sensitivity and specificity of a novel method of screening for cocaine in hair, based on MALDI-MS, have been evaluated.

Methods. The extraction procedure consists of shaking 2.5 mg of pulverized hair samples in the presence of an acidic solution and one stainless steel bullet, by an automatic pulverizer, for 5 min. 1 µL of the extract is deposed on a MALDI sample holder, previously scrubbed with graphite and, after dryness, the α-cyano-4-hydroxycinnamic acid (matrix) solution is electrosprayed

over the dried sample surface, by a sieve-based device, to achieve an uniform distribution of matrix crystals. The identification of cocaine was obtained by post-source decay experiments performed on its MH+ ion (m/z 304), with a limit of detection of 0.1 ng/mg of cocaine. A total of 47 samples (20 negative and 27 positive) were analyzed in parallel by MALDI and a reference gas chromatography-mass spectrometry method.

Results. The obtained results demonstrate specificity and sensitivity of 100% for MALDI. Evidence of cocaine presence was easily obtained even when hair samples, exhibiting particularly low cocaine levels (< 0.5 ng/mg), were analyzed.

Conclusions. The described MALDI method was proved to be of easy application and robust and lead to unequivocal results, suggesting that it can be proposed as a fast preliminary screening method for the determination of cocaine in hair.

Keywords: cocaine, hair, MALDI-MS, screening

O59. Liquid chromatography-ion trap-mass spectrometry assay for the simultaneous determination of methadone, cocaine, opiates and metabolites in human umbilical cord

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Introduction: Detecting drug exposure during pregnancy is the most effective way to improve pre- and post-natal care in the mother and newborn. Meconium is currently considered the specimen of choice for this purpose; however, new research suggested umbilical cord as an alternative to meconium for detecting fetal drug exposure.

Aims: The goal of our research was to develop and validate an analytical method for the simultaneous quantification of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine (BE), 6-acetylmorphine (6AM), morphine and codeine in human umbilical cord.

Methods: 1 g umbilical cord was homogenized with 5 mL 0.1% perchloric acid for 1-2 min. Samples were centrifuged after adding 50 µL of deuterated internal standards, and the supernatant collected for solid phase extraction using Strata XC mixed mode cartridges. Chromatographic separation was performed in a Synergi Polar-RP column (75 mm × 2 mm, 4 µm, Phenomenex®, Torrance, CA, USA), with a gradient of (A) 0.1% formic acid and (B) acetonitrile. Detection was performed in an ion trap mass spectrometer, using the most prominent MS2 transition for the quantification, and two additional MS2 or MS3 fragments for confirmation purposes, except for EDDP, for which only one MS3 fragment was available. Validation of the method included the study of linearity, limits of detection (LOD) and quantification (LOQ), intra- (n=5) and inter-assay imprecision (n=20) and analytical recovery (n=20), carryover, endogenous and exogenous interferences, hydrolysis, matrix effect, extraction and process efficiency, and stability of analytes under different conditions.

Results: All analytes eluted within 10 min with a total chromatographic run time of 15 min. Linearity was verified from 10-2000 ng/g for methadone and 2.5-500 ng/g for other analytes, applying a 1/x weighting factor, with coefficients of regression >0.99. Limits of detection (LOD) were between 0.25-2.5 ng/g. Intra and inter-assay imprecision were <15%, and intra- and inter-assay analytical recovery were >85% for all analytes. Carryover was <LOD. Selectivity was verified as no quantifiable endogenous interferences were observed in the analysis of 10 blank umbilical cords. In addition, specimens fortified with analytes of interest at low quality control concentrations and common licit and illicit drugs at 1000 ng/g, quantified within 85-115% of target with a CV<15% for all analytes. Matrix effect experiments showed suppression of signal between 4.9-29.9%, except for EDDP, for which a 39% enhancement was observed; however, this effect was compensated by the use of its deuterated analogue, which showed a similar effect. Percentage formation of BE and morphine after cocaine and 6AM hydrolysis were 0.19% and 1.87%, respectively. Extraction and process efficiency were >59.2% and 48.6%, respectively, for all analytes.

Conclusion: A method for quantification of methadone, EDDP, cocaine, BE, morphine, codeine and 6AM in umbilical cord was developed and fully validated, satisfying acceptance criteria. The method will be applied to the analysis of specimens following controlled administration of methadone in opioid-dependent pregnant women, and will contribute to the knowledge of in utero drug exposure.

Keywords: methadone, in utero drug exposure, umbilical cord, opiates, cocaine.

O60. Concentration profiles of methylphenidate in serum and oral fluid of children under medical treatment with an extended-release preparation

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Introduction: Oral fluid is considered to be a smart alternative to the more common blood or urine testing. In a preliminary study Marchei *et al.* concluded that oral fluid analysis is useful for monitoring of methylphenidate intake and to fit the dosage [1]. Oral fluid sample collection is non-invasive, and thus might be of special interest when working with children.

Aim: The aim of this project was to establish concentration ranges in oral fluid after a single oral dose of 20 mg methylphenidate (extended-release) to 24 children and to gain information about the variability of the saliva/serum coefficient of methylphenidate.

Methods: Oral fluid and blood were collected over 8 h according to the protocol of the clinical study. The samples were stored at -80 °C until sample preparation. 500 µL of each sample were extracted after addition of 20 ng D5-MDEA as internal standard using alkaline liquid-liquid extraction. The residue was reconstituted in 100 µL LC eluent, 20 µL were injected into the ESI-LC-MS/MS system, operated in the MRM mode.

Results: Methylphenidate concentrations were 1 – 35 ng/mL in serum and 1 – 110 ng/mL in oral fluid during 8 h after a single dosage. Oral fluid concentrations exceeded the LOD of 0.2 ng/mL approx. 0.5 hours after administration of methylphenidate in over 80% of the cases and stayed above 5 ng/mL for 8 hours after intake. Oral fluid concentrations as well as serum concentrations among the 24 probands were highly variable. Oral fluid/serum coefficients over the monitored period varied from 0.4 to 2.4. Therapeutic ranges in serum could be confirmed by positive oral fluid analysis in most cases – showing the utility of oral fluid analysis for monitoring compliance (after prescription) or abuse (without prescription).

Conclusions: This project provides information about concentration profiles of methylphenidate after a single dose of 20 mg methylphenidate (extended-release) and the saliva/blood coefficient of methylphenidate. Oral fluid analysis is a valuable tool for monitoring compliance or abuse of methylphenidate.

Reference: [1] Marchei E, Farrè M, Pellegrini M, Rossi S, García-Algar O, Vall O, Pichini S., Liquid chromatography-electrospray ionization mass spectrometry determination of methylphenidate and ritalinic acid in conventional and non-conventional biological matrices, J Pharm Biomed Anal. 2009; 49(2): 434-439.

Keywords: methylphenidate, therapeutic drug monitoring, oral fluid

O61. Screening of 17 benzodiazepines in artificial lachrymal fluids using liquid chromatography coupled to tandem mass spectrometry

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Aims: Tears are produced by blood filtration tacking place in lachrymal glands. They are mainly (more than 99%) made of water and salt (mostly sodium chloride). Some publications mention the use of this medium to quantify drugs in pharmacological studies (1,2,3). The main objective of this work is to extend the usage of this alternative matrix to toxicology. We have developed an identification method for 17 benzodiazepines or assimilated drugs in artificial lachrymal fluid using liquid chromatography coupled to quad tandem mass spectrometry. This technique was then applied on patient from an ophthalmology centre.

Method: A mix solution of 17 benzodiazepines and their deuterated homologues was done using artificial lachrymal fluids. Several therapeutic (5-900 ng/mL according to molecules) and toxic (50-5000 ng/mL according to molecules) range specimens were created and then put down on a blotting paper strip used in ophthalmology. When dried done an alkaline liquid/liquid extraction was done using a quaternary organic solvent mix. Reconstituted extracts where then separated using an Acquity UPLC™ HSS C18 column in gradient elution mode with ammonium formate (pH 3,5) and acetonitrile. Total cycle time is 15 minutes. The instrument platform is made of an Acquity UPLC™ system coupled to a Waters® Quattro micro™. Patients tears collection was done using a dedicated blotting paper strip sterile and with a volume based scale. Collection is done by positioning the strip at the inferior one/third of the external inferior conjunctival dead end and let them collect tears during 5-10 minutes.

Results: All 17 benzodiazepines were detected at the studied concentrations. The responses were linear (correlation factor >0.99) to concentration (5-2500 ng/mL) for all tested benzodiazepines. Reproducibility CVs were better than 20% for all tested concentrations. Patient's tears analysis showed the presence of several benzodiazepines confirmed by the ophthalmologist patient's questionnaire.

Conclusion: The use of a LC-MS/MS applied to tears analysis lead to quick, easy, sensitive and specific methods. It was possible to detect all tested benzodiazepines. Additional work is needed to build a correlation between concentrations of free drugs fraction in blood with the tears ones.

References:

1. Nakajima M. et coll. Assessment of Tear Concentrations on Therapeutic Drug Monitoring. III. Determination of Theophylline in Tears by Gaz Chromatography / Mass Spectrometry with Electron Ionization Mode. Drug Metab. Pharmacokin. 2003; 18(2): 139-145 ; 2. Arnold DR. et coll. Quantitative determination of besifloxaxin, a novel fluoroquinolone antimicrobial agent, in human tears by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2008; 867: 105-110; 3. Tian D. et coll. Liquid chromatography-mass spectrometry method for determination of thiamphenicol in rabbit tears. J Pharm Biomed Anal. 2008; 48: 1015- 1019.

Keywords: tears, alternative matrices

O62. On-line desorption of dried blood spot: a novel approach for the direct LC/MS analysis of µ-whole blood samples

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Introduction: Since 1960, the filter paper 903 S&S produced by Whatman has been widely used as support for collecting newborns' capillary blood for the screening of neonatal metabolic disorders. Called dried blood spots (DBS), this procedure offers the advantage of being less invasive and more cost effective in terms of sample collection, shipment and storage as compared to venipuncture. Recently the DBS sampling has been applied to the monitoring of pharmaceutical compounds including antimalarials, antiepileptics, antiretrovirals, antibiotics, and immunosuppressive drugs. However, one weakness of the procedure is the off-line extraction step: compounds need to be extracted from the filter paper before analysis.

Aim: The aim of this work is to present a new concept, called on-line desorption of dried blood spots (on-line DBS), allowing, without any pretreatment, the direct analysis of a dried blood spot coupled to liquid chromatography mass spectrometry device (LC/MS).

Methods: The system is based on an inox cell which can receive a blood sample (10 µL) previously spotted on a filter paper. The cell is then integrated into LC/MS system where the analytes are desorbed out of the paper towards a column switching system ensuring the purification and separation of the compounds before their detection on a single quadrupole MS coupled to atmospheric pressure chemical ionisation source (APCI).

Results: To demonstrate the applicability of the concept, saquinavir, imipramine, and verapamil were chosen as model compounds. Despite the use of a small sampling volume and a single quadrupole detector, on-line DBS allowed the analyses of these three compounds over their therapeutic concentrations from 50 to 500 ng/mL for imipramine and verapamil and from 100 to 1000 ng/mL for saquinavir. Moreover, the method showed good repeatability with relative standard deviation (RSD) lower than 15% based on two levels of concentration (low and high). Function responses were found to be linear over the therapeutic concentration for each compound and were used to determine the concentrations of real patient samples for saquinavir. Comparison of the values obtained with on-line DBS procedure with those of a validated LC/MS/MS method used routinely in a reference laboratory showed a good correlation between the two methods. Moreover, good selectivity was observed ensuring that no endogenous or chemical components interfered with the quantitation of the analytes.

Conclusion: This work demonstrates the feasibility and applicability of the on-line DBS procedure for bioanalysis.

Keywords: on-line DBS; LC/MS; whole blood; column switching

O63. The detection and quantification of lorazepam and its phase II metabolite in fingerprint deposits by LC-MS/MS

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Introduction: The use of fingerprints as an alternative biological matrix for testing for the presence of drugs and/or their metabolites is a novel area of research in analytical toxicology. In all cases regarding analysis of fingerprints for drugs, either the parent compound or a major phase I metabolite has been exclusively targeted, no investigation having analysed for the presence of phase II metabolites.

Aim: The quantitative analysis for the benzodiazepine lorazepam and its 3-O-glucuronide conjugate in fingerprints following administration of the drug (2 mg) to volunteers was investigated.

Methods: Fingerprints were deposited on glass cover slips prior to drug administration (0 h) and then at 2, 4, 6, 8, 12, 24 and 36 h post-drug administration. For analysis, glass cover slips were immersed in a solution of dichloromethane:methanol, containing tetradeuterated lorazepam as an internal standard. Following sonication and shaking, the solvent was transferred, evaporated to dryness and the residue reconstituted into mobile phase for analysis by liquid chromatography-tandem mass spectrometry. Chromatography was achieved using a reverse phase (C18) column for analysis of lorazapem and its glucuronide, and a hydrophilic interaction column (HILIC) for the analysis of creatinine. For quantification

purposes, calibrants of the target analytes, were prepared in the mobile phase containing the same internal standard.

Results: Lorazepam and its glucuronide were only detected where 10 prints had been combined, up to 12 h following drug administration. In every case, the amount of lorazepam glucuronide exceeded that of lorazepam, the peak amounts being 210 pg and 11 pg respectively. Adjusting for creatinine smoothed the elimination profile.

Conclusion: To our knowledge, this represents the first time a drug glucuronide has been detected not only in skin secretion but also deposited fingerprints. Currently, further investigations are being performed concerning the analysis of other psychoactive drugs in fingerprint residues, including the use of antibody-nanoparticle conjugates, in conjunction with LC-MS/MS, for screening and confirmatory purposes.

Keywords: fingerprint, HILIC-MS/MS, lorazepam glucuronide, quantitative analysis

O64. Prenatal tobacco exposure, meconium nicotine biomarkers and infant outcomes

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Introduction: Approximately 18% of pregnant women continue to smoke during pregnancy despite health warnings. Tobacco biomarker testing of meconium, the first neonatal feces, can determine if prenatal exposure occurred, but it is unclear if quantitative nicotine and/or metabolite concentrations predict neonatal outcomes.

Method: 62 women provided extensive tobacco use histories each trimester; cotinine oral fluid tests corroborated maternal self-report. Meconium nicotine, cotinine and trans-3'-hydroxycotinine concentrations were determined. In another infant cohort, these three analytes improved tobacco exposure detection in meconium by 25%, as compared to cotinine alone.

Results: Of 62 women, 13 denied smoking, corroborated by negative maternal oral fluid and neonatal meconium results. An additional 6 women reportedly quit smoking during the first or second trimester; however, 3 meconium specimens were positive for nicotine biomarkers. Of these, 2 women had positive oral fluid tests in the third trimester, indicating recent cigarette use, and the third woman lived with a smoker. The other 43 women smoked throughout pregnancy; 88.4% had positive nicotine biomarkers in meconium. Oral fluid and meconium testing identified cocaine, opiate and cannabis consumption among admitted tobacco smokers, but not nonsmokers or quitters. No other demographic differences existed for nonsmokers, quitters and smokers. Meconium cotinine, trans-3'-hydroxycotinine and total tobacco biomarker concentrations were significantly correlated to total cigarettes consumed during the second and third trimesters; cotinine and total biomarkers were related to third trimester cigarettes. Among women smoking similar numbers of cigarettes, living with a partner who also smoked did not significantly affect meconium concentrations. Meconium nicotine biomarker concentrations were not correlated to gestational age, birth weight, length, head circumference or Apgar scores, although significantly decreased head circumference was observed when a tobacco marker was present.

Conclusion: Meconium analysis confirmed maternal self-reported tobacco use in over 87% of participants, a much higher rate of confirmation than previously reported for prenatal methamphetamine or cannabis exposure. Tobacco-using women also were more likely to consume cannabis and other drugs than non-smokers. Tobacco biomarkers in meconium were associated with decreased head circumference, but no concentration-outcome relationships were noted.

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O65. Munchausen syndrome by proxy. A special forensic toxicological analysis challenge

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Introduction: Munchausen syndrome is a factitious disorder, that is a condition in which a person acts as if he or she has an illness by deliberately producing, feigning, or exaggerating symptoms. When a person suggests that an individual he or she is caring for has an illness when this individual is not really sick, then it is termed Munchausen syndrome by proxy (MSP). MSP is most commonly associated with adults feigning the illness in a child. Tthis is considered to be a form of child abuse. The authors report on a case of suspected MSP where the mother induced real symptoms of ataxia in her 4 children (aged 9, 5, 3 and 2 years of age).

Patients and methods: Initial examination at the hospital by a pediatrician failed to explain the observed symptoms (acute ataxia) of the 2 older children, but immunoassay analysis of the urine provided good clues that benzodiazepines and opiates had been administered. These screening positives could not be verified immediately at the hospital thus it was fortunate that the MD notified the cases to the public prosecutor who subsequently requested toxicological investigations to be undertaken on blood, urine and hair of the 4 children on samples obtained 2 days after their admission to hospital. These were submitted to our specialist forensic laboratory and the results where reported to the prosecutor within 2 days. We were then also asked to investigate samples obtained from the parents. The blood and urine samples were analysed using GC-MS and LC-DAD according to our procedure for the determination of general unknown drugs in body fluids. Segmental hair samples (3 × 2 cm) were analysed using a combination of GC-MS, LC-DAD (general unknown screening) and by LC-MS/MS (low level screening).

Résults and Discussion: 1) Children: Nordiazepam was found at toxic concentrations in the blood of the 3 older children (respectively 3.9, 2.1, 3.5 ng/mL), together with noticeable amounts of oxazepam and prazepam. Codeine and paracetamol were also detected in the blood at therapeutic concentrations. The results for the three 2 cm-segments (Sn) of hair for the 4 children (CHn) are reported (in ng/mg) in the following table:

		CH#1			CH#2			CH#3			CH#4	
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Prazepam	ND	177	1.7	71	70	65	313	278	319	0.2	0.1	0.1
Nordiazepam	408	252	107	486	132	50	371	93	115	0.1	0.1	0.1
Oxazepam	34	11	<2	57	9.5	2.8	63	7.8	2.9	+	+	+
Codeine	1.1	0.5	0.25	1.1	0.2	0.1	3.8	1.1	0.8	ND	ND	ND
Paracetamol	1.7	1.0	1.7	<1	<1	<1	<1	<1	<1	6.8	NP	NP
Oxomemaz	1.8	ND	ND	3.1	ND	ND	2.9	ND	ND	4.4	3.6	4.1

Our findings demonstrated that 3 of the 4 children had been given large amounts of prazepam plus therapeutic doses of codeine, paracetamol and oxomemazine on a regular basis over a period of approximately 6 months with lower doses of prazepam, paracetamol and oxomemazine being administered to the fourth child. We also confirmed that even 48 hours after their admission to hospital the children still had concentrations of nordiazepam that lay within the toxic range.

2) Parents: Analyses of the father's hair suggested that he was an occasional user of prazepam whilst analysis of the mother's hair suggested that she was regularly using high doses of prazepam and therapeutic doses of fluoxetine, tramadol and tetrazepam. The mother was reported to have had 60 appointments with doctors in a 2 month period. These appointments had been alone and with her children for their alleged "health problems". She had received a number of prescriptions notably for a total of 1600 mLs of codeine syrup and 560 tablets of prazepam.

Conclusion: All the indicators for MSP were present but they were only recognized after we provided our analytical results (these included that the

mother was involved in a health-care field, the children had non-classical children's symptoms, the doses of medications were increasing with time). This report demonstrates the benefits of employing an adequately qualified and highly experienced forensic laboratory with a broad spectrum of analytical methods in blood and hair in solving cases with a potential forensic background.

O66. The minimal dosage of ketamine and triazolam detectable in hair after administration

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Introduction: Hair was suggested as a valuable specimen in the drug-facilitated crime cases. But much research still needs to be performed on how to interpret the results.

Aims: The purpose of this study was to investigate the minimal dosage of ketamine and triazolam detectable in guinea pig hair after a single dose administration.

Methods: A sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method has been developed for the determination of ketamine, triazolam and their major metabolites, norketamine and α-hydroxytriazolam in hair. The hair sample preparation involved decontamination, acid hydrolysis and a liquid–liquid extraction. Deuterated ketamine and deuterated triazolam were used as internal standards. The analysis was performed by positive electrospray ionization, and multiple reactions monitoring (MRM) mode was used.

Results: The limit of detections (LOD) for ketamine and triazolam were 0.5 and 1.0 pg/mg respectively when approximately 20 mg hair material was processed. After shaving back hair (4 cm × 3 cm), ketamine or triazolam were administered intraperitoneally to guinea pigs at three doses (low, medium, and high), respectively. And hair samples in different colors were collected separately on the 7th and 14th day postdose, although it turned out only those collected in the first week could be detected. At the dosage of 0.1 mg/kg, ketamine was detected in 11 out of 18 (positive detection rate 89%) hair segments collected. Norketamine, with a LOD of 1 pg/mg, was detected only in 8 of these segments (positive detection rate 44%). For the higher dosage of 1 mg/kg, ketamine and norketamine were detected in all the segments in the first week. Following controlled administration of 0.1 mg/kg dosage, 69% of specimens had detectable triazolam with LOD of 1 pg/mg hair. At a higher dose of 0.5 mg/kg, both triazolam and its metabolites, α-hydroxytriazolam, were detected in all the segments in the first week.

Conclusions: 0.1 mg/kg was the minimal dosages of ketamine and triazolam detectable in hair using LC-MS/MS. Such studies provide basic data that will be useful in the application of hair analysis in the drug-facilitated crimes and in the interpretation of results. Assays with lower detecting limits may decrease the minimal dosages.

Keywords: ketamine, triazolam, the minimal dosage, hair, LC-MS/MS, guinea pig

O67. Fast and simple liquid-liquid extraction method for LC-MS/MS quantification of 140 analytes from different drug classes in human plasma

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Introduction: Multi-analyte procedures covering a wide range of drug classes should be integrated in routine work of drug monitoring as well as clinical and forensic toxicology. Different workup procedures are often used for different drug classes. Having only one extraction procedure covering all drug classes should make routine and emergency toxicology less complex, faster, and cheaper.

Aim: The aim of the present study was to develop a simple extraction procedure of human plasma for the quantification of about 140 analytes from different drug classes, *i.e.* antidepressants, neuroleptics, benzodiazepines, beta-blockers, sulfonylurea-type antidiabetics, and drugs relevant in the context of brain diagnosis.

Methods: Different solid-phase (BondElute HCX, Isolute C18, Strata-X 33 µm, and 3M Empore C8) and liquid-liquid extraction methods (butyl acetate, butyl acetate/ethyl acetate, diethyl ether/ethyl acetate) were tested. The pre-selected liquid-liquid extraction method with butyl acetate was further modified varying solvent volume and composition. Furthermore, extraction at different plasma pH values and after protein precipitation was also tested. Recovery and matrix effects tests were evaluated for the final extraction procedure at two different analyte concentrations (low and high) with plasma samples from six different sources. Recovery results calculated by using peak areas were compared to those calculated by area ratios to an internal standard (IS) spiked before extraction. The final extraction procedure was as follows: 600 µL ethyl acetate/butyl acetate (1/1) added to 0.5 mL plasma, shaken for 2 min on a rotary shaker and centrifuged for 3 min at 10,000 g. Finally, 500 µL of the supernatant were transferred to an autosampler vial, evaporated under a gentle stream of nitrogen and reconstituted in 50 µL acetonitrile/10 mM ammonium formate buffer (1/1). All extracts were analyzed with an Accela UHPLC coupled to a TSQ Quantum Access triple-quad mass spectrometer (ESI+, MRM mode, Thermo Fisher Scientific, Dreieich, Germany).

Results: Initial experiments showed that liquid-liquid extraction with butyl acetate covered most analytes but with in part insufficient recoveries. Variation of plasma pH values or solvent volume lead to increased recovery and/or reproducibility for many but not all of the tested drugs. However, with a mixture of ethyl acetate/butyl acetate (1/1) the following recoveries (median, ratio) were obtained at low and high concentration levels, respectively: for antidepressants 68% and 62%, for neuroleptics 69% and 74%, for benzodiazepines 75% and 71%, for beta-blockers 52% and 56%, for antidiabetics 41% and 44%, for analytes relevant in the context of brain death diagnosis 71% and 74%. With the exception of 24 analytes, all compounds showed recoveries with acceptable variability ≤15% and ≤20% CV. Recovery results obtained by comparing peak areas were nearly the same as shown above but 70 (low level) and 38 (high level) analytes lay out of range with 25% CV and 26% CV, respectively. Matrix effects with more than ≤20% difference of the median value were observed for 35 analytes.

Conclusions: With this fast and simple liquid-liquid extraction of 0.5 mL plasma with 600 µL ethyl acetate/butyl acetate 116 of the 140 analytes could be extracted with suitable recovery and acceptable variability using an IS for compensating fluctuations during analysis.

Keywords: liquid-liquid extraction, LC-MS/MS, quantification, multi-drug, plasma.

O68. General unknown screening of illicit drugs: a novel approach using high resolution and accurate mass

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Introduction: In recent times LC/MS has become the technology of choice for screening of illicit drugs. Two main approaches for tandem MS have been used in this area. The first one is called MTS1: muti-target-screening and the second one GUS2: general unknown screening. In both cases, these two approaches are limited by the number of entries available in the MS2 library.

Aim: In this work, we will present a completely new approach based on mass accuracy. Confirmation is made using accurate mass detection of the

analyte (below 5 ppm) and its retention time. Examples of data from real samples will be presented and extra parameters used for confirmation will be discussed.

Methods: Plasma samples were prepared using liquid/liquid extraction with ToxiTube A (Varian). The HPLC separation was performed on a Hypersil Gold PFPTM 150 × 2.1 mm, 5 µm. Screening and detection was carried out on an Exactive BenchTop LCMS OrbiTrap MS. Acquisition was in full scan mode with resolution of 100,000, and further, HCD (higher energy collision induced dissociation) was utilised to obtain fragmentation data. Data were processed using ToxIDTM software.

Results: In this work, we have evaluated a new GUS approach using the Exactive instrument. Data were acquired using full scan MS at high resolution (R = 100.000) and fragmentation spectra were collected using HCD. The data were processed through ToxID™ software. This processing software provides results based on the accurate mass of the parent ion and the retention time. Currently, over 300 compounds have been evaluated using this approach and real plasma samples have been analysed in order to evaluate the robustness of the method. The results obtained via ToxID are further confirmed by using the accurate mass of fragment ions obtained during HCD. Moreover, comparisons between the experimental isotopic distribution and the theoretical values have been performed.

The high scan rate capability of the instrument is shown to be compatible with the narrow peak widths observed in ultra high performance chromatography (UHPLC) analyses, and thus, we have been able to reduce the total LC run time from 30 to 15 min.

In this approach, the screening is no longer limited to the number of tandem MS spectra entries available in the library and the accurate mass ion list can easily be extended to thousands of compounds including illicit drugs, pesticides and pharmaceutical drugs.

Conclusion: High resolution, mass accuracy and fast scan rate capabilities make the exactive a good approach for general unknown screening.

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Keywords: general unknown screening, serum, illicit drugs, mass accuracy

O69. Development and validation of a drugs screening method in biological matrices using an hybrid LC-MS/MS system

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Introduction. A multi-target drugs screening (MTS) method in biological matrices based on LC-MS/MS was developed. A small particle size C18 column (1.9 µm) was used for the chromatographic separation, providing very narrow peaks with high resolution and allowing an excellent separation of most of the analytes in 12 minutes.

Aim The purpose of this new method of MTS including a rapid resolution chromatographic separation was to show that LC-MS/MS with MRM, is able to screen for a significantly large number of compounds and to provide a valuable alternative to the Remedi® (BioRad) instrument.

Methods. Serum and urine samples containing deuterated internal standards were prepared by SPE. The extract was injected onto an Hypersil Gold analytical column (100 × 2.1 mm, 1.9 µm; ThermoScientific). Mobile phase A was water with 0.1% formic acid and sodium formate 1 µM and mobile phase B was acetonitrile. Rapid separation chromatography (Agilent 1200 HPLC) was achieved using a gradient elution from 95:5 ramping to 30:70 (A:B) over 12 minutes. An information-dependent acquisition (IDA) experiment in MS/MS on a QTRap (AppliedBiosystems/MDS Sciex, Ontario, Canada) instrument was used. In the IDA experiment, the MS detection method included a multiple reaction monitoring (MRM) as survey scan and an enhanced production ion (EPI) scan as dependant scan. Drug identification was carried out by a library search using SmileMS, a newly developed software (GeneBio, Geneva, Switzerland) based on EPI spectra similarity. SmileMS is a web-based client-server software platform. The graphical web interface includes a number of intuitive features to launch searches and explore results at various levels of details (list of chemical compounds, spectral interpretation views, annotated chromatogram and global reports).

Results. The results observed during the method evaluation confirmed the influence of the sample matrix (matrix effect). Therefore, an adapted sample preparation as well as a good chromatographic separation were mandatory to obtain good results. Separation of benzodiazepines, antidepressive and neuroleptics agents which are the main drugs involved in intoxication cases was optimised to offer sufficient detection capability in terms of limit of detection and process efficiency. The method showed good performance of first importance such as total chromatographic time of 15 min with preserved resolution allowing an automatic peak detection and compound identification. The process efficiency (PE) was excellent (>90%) for 32% of the tested compounds, good for 33% of these compounds (PE: 70 – 90%) and for the other compounds results were variable but acceptable regarding the analytical sensitivity and the toxic concentration.

Conclusion. The described methodology allows the identification of the main drugs incriminated in intoxications. The procedure is robust, sensitive and selective enough. Therefore, this new screening method is a reliable alternative to the Remedi® instrument as a first-line screening in emergency clinical toxicology.

O70. Therapeutic drug monitoring of 14 antiretroviral drugs using LC-MS

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Background: Prospective and retrospective studies have provided evidence of the clinical and virological benefit of incorporating TDM into routine patient care. Because antiretroviral therapy consists always of a combination of different drugs, analysis can be simplified if different drugs are measured at the same time. Therefore, LC-MS or LC-MS/MS is nowadays the analytical method of choice.

In 2003 we have published an LC-MS method (1) for the quantification of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir after solid-phase extraction. In the meantime atazanavir, darunavir, tipranavir, raltegravir, etravirin and maraviroc have been introduced into the marked.

Aim: The purpose of this work was to include all antiretroviral drugs belonging to the classes of proteinase inhibitors and non-nucleoside reverse transcriptase inhibitors as well as the integrase inhibitor and the entry inhibitor in our LC-MS procedure. This would then enable the treating physicians to monitor all antiretroviral drugs which may be subject of pharmacokinetic interactions. In the process of including them in our analytical procedure an additional aim was to reduce the sample volume, to simplify the sample preparation and to shorten the chromatographic run time.

Method: Sample preparation consisted in the addition of a solution of the internal standard (proteinase inhibitor analogue) in a mixture of methanol, 0.1 M ZnSO4 and acetonitrile to 100 µl serum which resulted in protein precipitation. After centrifugation, the supernatant was diluted with the aqueous elution buffer before injection into the HPLC system. The different drugs were analyzed by reversed-phase chromatography and detected by mass spectrometry after positive atmospheric pressure chemical ionization (APCI) using an LCQ Fleet mass spectrometer (Thermo Fisher Scientific, Reinach).

Results: Depending on the target concentrations in patients, the calibration curves of the new method were linear in the range of 0.01 – 30.0 mg/l. The limit of quantification was accordingly between 0.01 and 0.3 mg/l. The imprecision was < 10% and the accuracy 92 – 108%. The absence of ion suppression has been demonstrated for the analysis of all compounds.

Conclusions: The performance data of the described LC-MS method as well as the results of the external quality control scheme (KKGT, the Netherlands) demonstrate that this simplified method allows the quantification of 14 different proteinase inhibitors, non-nucleoside reverse transcriptase inhibitors, the integrase inhibitor and the entry inhibitor in heparine plasma of patients with HIV infection.

Reference: (1) Rentsch, KM. J Chromatogr B, 2003; 788: 339.

Keywords: amprenavir, efavirenz, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, atazanavir, darunavir, tipranavir, raltegravir, etravirin, maraviroc, LC-MS, heparine plasma

O71. Workup of urine samples for systematic toxicological analysis using GC-MS: acid hydrolysis/liquid-liquid extraction/acetylation versus miniaturized enzymatic hydrolysis/liquid-liquid extraction/acetylation

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Introduction: Liquid-liquid extraction of urine samples after acid hydrolysis and acetylation has been a versatile and fast sample workup for GC-MS-based STA in the Department of Experimental & Clinical Toxicology in Homburg (Saar), Germany. The procedure was developed many years ago when GC-MS apparatuses were much less sensitive than nowadays. Hence comparatively large urine volumes (5 mL) and solvent volumes (5 mL) were required. Moreover, acid hydrolysis is rapid but may lead to formation of artifacts.

Aims: The aim of the present study was therefore to compare the performance of a similar workup procedure, but with enzymatic hydrolysis instead of acid hydrolysis as well as sample and solvent volumes reduced by a factor of ten, against the original procedure.

Methods: Forty-two urine samples were worked up according to the established procedure: 5 mL urine, one half submitted to acid hydrolysis, buffered (pH 8-9) with 2 mL of aqueous ammonium sulfate/1.5 mL of aqueous sodium hydroxide, extracted with 5 mL of ethyl acetate-dichloromethane-isopropanol (3:1:1 v/v/v), organic phase evaporated and acetylated under microwave irradiation, reconstitution in 100 µL of methanol. The derivatized extracts were analyzed by GC-MS (Agilent, HP 5890 Series II gas chromatograph combined with a HP 5972A MSD mass spectrometer). The same samples were also worked up according to the new protocol: 0.5 mL urine, buffered to pH 5-6 with acetate buffer (100 µL, 2 M), submitted to hydrolysis with glucuronidase/arylsulfatase from Helix pomatia for 15 minutes at 50 °C, buffered to pH 8-9 with 0.2 mL phosphate buffer, extracted with 1 mL of ethyl acetate-dichloromethane-isopropanol (3:1:1 v/v/v), organic phase evaporated and acetylated under microwave irradiation, reconstitution in 50 µL of methanol. The derivatized extracts were analyzed by GC-MS (Shimadzu GCMS-QP2010 Plus gas chromatograph mass spectrometer). The results were compared to those obtained with the established procedure.

Results: Despite the much lower sample and solvent volumes for the new workup procedure, there were only very few differences in the spectrum of analytes detected by both methods. With exception of some benzodiazepines, all compounds identified with the established method were also identified with the new method. In the case of the benzodiazepines lorazepam and diazepam/oxazepam, artifacts were detected with the new method rather than the benzophenones resulting from acid hydrolysis in the established procedure. In one case diazepam and in another case lorazepam could not be detected with the new procedure, although they had been found with the established method. However, the respective urine samples contained only small amounts of these benzodiazepines.

Conclusion: The presented data show that using modern-day GC-MS apparatuses allow considerable reduction of sample and solvent volumes which is advantageous when only small amounts of urine are available. It further shows that enzymatic hydrolysis for 15 minutes at elevated temperature is an alternative to aggressive acid hydrolysis and should be sufficient for emergency toxicology. However, one must be aware that compounds present in low concentrations, especially benzodiazepines, may be overlooked with the new procedure.

Keywords: GC-MS, urine, workup

O72. Normetabolites of hydrocodone, oxycodone and codeine are useful biological markers for interpretation of drug source in pain patients

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Introduction: Opioid medications are the cornerstone of pain management; some 90% of chronic pain patients are currently being prescribed these drugs. Urine drug testing of pain patients provides objective information to health specialists regarding patient compliance, diversion, and concurrent illicit drug use. Interpretation of urine test results for semi-synthetic opiates can be difficult because of complex biotransformations of parent drug to metabolites that also are available commercially and may be abused. For example, hydrocodone (HC) is a commercial analgesic that is frequently prescribed for pain relief. HC is metabolized and excreted in urine as numerous metabolites including hydromorphone (HM), dihydrocodeine (DHC), and norhydrocodone (NHC). Urine test panels frequently include HC, HM, and DHC, but not NHC. The metabolites, HM and DHC, are available commercially and also may be abused. Consequently, for a patient prescribed HC, a positive urine test for HM in the absence of HC could be interpreted as either HC use or HM abuse. However, a positive test for NHC, even in the absence of HC, would be indicative of HC use. Similarly, detection of normetabolites of other opiate-related drugs can be useful in interpretation of urine tests.

Aim: The goal of this study was to evaluate the prevalence and patterns of three normetabolites, NHC, noroxycodone (NOC) and norcodeine (NCOD), in urine specimens of pain patients prescribed HC, oxycodone (OC), or codeine (COD) and/or other medications.

Methods: Urine specimens from pain patients undergoing chronic opioid therapy were analyzed by liquid chromatography tandem mass spectrometry (LC-MS-MS) for the presence of COD, NCOD, morphine (MOR), HC, NHC, HM, DHC, OC, NOC, and OM. The Limit of Quantitation (LOQ) for these analytes was 50 ng/mL. The study was approved by an Institutional Review Board.

Results: Of the total specimens (n=2654) tested, 71.4% (N=1895) were positive (≥LOQ) for one or more of the analytes. The prevalence (N) of

positive results for HC, OC and COD were 694, 960, and 32, respectively. The number of specimens positive for normetabolites in combination with parent drug was as follows: HC, 527; OC, 732; and COD, 9. The number of specimens positive for normetabolite in the absence of parent drug was as follows: NHC, 59; NOC, 100; and NCOD, 3. Thus, 8.5% of all positive specimens contained normetabolite in the absence of parent drug. For specimens that were negative for parent drug and positive for normetabolite, some contained another metabolite of the parent drug and some were negative for other metabolites. For example, 21 specimens were positive for NHC and HM, 3 specimens were positive for NHC and DHC, 62 specimens were positive for NOC and OM and 1 specimen was positive for NCOD and MOR. The number of specimens positive for normetabolite and negative for parent drug and other metabolites were as follows: NHC, 35; NOC, 38; and NCOD, 2.

Conclusion: Inclusion of the normetabolites of HC, OC, and COD in urine test panels for pain patients improves detection rates by avoidance of false negatives (specimens positive only for normetabolite) and facilitates interpretation of which drug was consumed.

Keywords: opiates, urine, normetabolites

O73. Acute propylene glycol ingestion treated with fomepizole and haemodialysis

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Introduction: Propylene glycol (PG) (1,2-propanediol) is widely used as a solvent in different pharmaceutical products and as an ingredient of some antifreeze fluids. Because it is thought to have low toxicity, it has replaced ethylene glycol in numerous applications. But despite its low toxic potential, some severe intoxications have been reported following PG ingestion or following continuous intravenous injection of medications containing highly concentrated PG as a vehicle.

Case history: We report here the case of a 65-year-old women with chronic renal failure treated by peritoneal dialysis who accidentally ingested 200 g of PG. To improve efficiency of her peritoneal dialysis she was prescribed polyethylene glycol (PEG) preparation but her pharmacist erroneously delivered 2 litres of PG instead. After drinking 200 mL of liquid, complaining of burning sensations in the oesophagus, she called her pharmacist who recommended admission to the emergency room. She presented to her local hospital 90 min after ingestion with blood pressure at 150/80 mmHg, pulse rate at 75/min and a body temperature of 37 °C. She was conscious with neurological signs of inebriety. Biology showed a mild compensated metabolic acidosis with lactic acid concentration at 3.2 mmol/L and an osmolal gap of 131 mOsm/kg. Worsening of neurological signs prompted medical staff to introduce a treatment by fomepizole, and haemodialysis was started on the basis of the pre-existing chronic renal failure. After a second session of haemodialysis osmolal gap decreased to 20 mOsm/kg and neurological signs of inebriety disappeared. On hospital day 2 the woman was transferred from intensive care unit to the nephrology department without adverse outcome.

Methods: Five samples were sent to the toxicology laboratory of Poitiers in order to analyse the PG concentration. Two plasma specimens have been sampled 3 and 5 hours after ingestion, another one just after haemodialysis and a last one just before the transfer in the nephrology department. A last specimen consisted in the haemodialysis liquid sampled after the end of the first session. The five specimen have been analysed by GC-MS after derivatization with phenylboronic acid. 1,3-propanediol was used as an internal standard.

Results: The first samples analysed showed PG concentrations of 7.4 and 8.5 g/L respectively 3 and 5 hours after ingestion. The high concentration of PG in the haemodialysis liquid (6.9 g/L) revealed the efficiency of this treatment. After the haemodialysis session, the PG concentration in plasma was 1.7 g/L and before discharge to the nephrology department the last sample showed a PG concentration of 0.7 g/L.

Conclusion: Despite its low toxicity, fatalities related to PG intoxication can occur. Patients with hepatic or renal failure are at increased risk of toxicity due to the accumulation of toxic metabolites. We report here the case of a woman with chronic renal failure successfully treated with fomepizole and haemodialysis after a massive PG intoxication taken at early stage. To the best of our knowledge this is the first report of a PG intoxication treated with fomepizole the competitive inhibitor of alcohol dehydrogenase. The mild metabolic acidosis observed and minimal lactate elevation are consistent with fomepizole-related inhibition of PG metabolism.

Keywords: propylene glycol, ingestion, haemodialysis, fomepizole

O74. Comparison of bias/precision- and beta-expectation interval-based accuracy evaluation for six-point and one-point calibration – a retrospective analysis of six validated assays

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Introduction: In bioanalytical method validation, bias (systematic errors) and precision (random errors) are generally evaluated separately with ±15% of the target value (±20% near LLOQ) and 15% RSD (20% near LLOQ), respectively, being considered acceptable (15-20 rule). However, in recent years an alternative approach based on so-called beta-expectation tolerance intervals representing a combination of systematic and random errors has become increasingly popular. The latter approach provides information on which percentage of future quality control (QC) measurements may be expected within pre-defined tolerance intervals.

Aim: The aim of the present study was to compare the 15-20 rule with the tolerance interval-based approach with particular respect to data obtained with six- and one-point calibration.

Methods: Bias and precision data were taken from a previous study with six validated assays for plasma analysis (Peters and Maurer, Anal Chem, 2007): three GC-MS methods for seven drugs relevant in brain death diagnosis (assay I), MDA, MDMA, and MDEA enantiomers (II), 18 amphetamine- and piperazine-derived designer drugs (III); three LC-MS methods for 15 neuroleptics and three of their metabolites (IV), 22 beta-blockers (V), 23 benzodiazepines, three Z-drugs, and flumazenil (VI). The data were used to calculate 95% beta-expectation intervals for full six-point calibration and one-point calibration with a calibrator close to the center of the full calibration range. As recently recommended by the GTFCh, the results were considered acceptable when lying within a tolerance interval of ±30% of the respective target values (±40% near the LLOQ). The results for six-point and one-point calibration were compared with each other and with the respective results obtained with the 15-20 rule.

Results: Using six-point calibration, all analytes of assays I and II fulfilled the 15-20 rule as well as the beta-expectation interval-based criteria. For assay III, IV, V, and VI the data for six, five, three, and four analytes, respectively, were outside the acceptance limits using beta-expectation intervals which would have been accepted with the 15-20 rule. The widest beta-expectation intervals despite fulfilled 15-20 rule were 60.3 to 23.1% at low concentrations (clozapine-N-oxide, assay IV), 31.7 to 38.3% at medium concentrations (MDBP, assay III), and 40.0 to 33.1% at high concentrations (mCPP, assay III). Using one-point calibration, one analyte in assay II (S MDA, bias 22.3%) and one in assay VI (nordazepam, -15.3% and -19.8% bias) failed the criteria of the 15-20 rule but fulfilled those of the beta-expectation interval approach. For assays I, III, IV, V, and VI the data for two, three, six, twelve, and six analytes, respectively, were outside the acceptance limits using beta-expectation intervals which would have been considered

acceptable with the 15-20 rule. The widest beta-expectation intervals despite fulfilled 15-20 rule were 38.1 to 55.5% at low concentrations (talinolol, assay V), 40.9 to 38.1% at medium concentrations (esmolol, assay V), and 13.0 to 41.4% at high concentrations (9-HO-risperidone, assay IV).

Conclusion: Even in bioanalytical methods fulfilling the widely used 15-20 rule single measurements considerably deviating from the target value and hence causing problems in routine QC are not unlikely, especially if precision and bias values are close to the respective acceptance limits. Using the beta-expectation interval approach with a tolerance interval corresponding to routine QC limits, methods associated with a high risk of causing problems in routine QC can be more effectively identified during validation than with the 15-20 rule.

Keywords: beta-expectation interval, tolerance interval, bias, precision, validation

O75. Total error for the validation of bioanalytical methods

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Introduction: Consistent and efficient use of any analytical procedure requires the knowledge of its reliability prior to its use. It is therefore necessary for each laboratory to validate their analytical methods. Validation is not only required by regulatory authorities [ICH, FDA, GxP] or in order to access accreditation [ISO 17025], but is also the ultimate phase before the routine use of the method. Analytical method validation must bring confidence to the laboratories in the results that will be generated since they are used to make critical decision. However, very little information is included about the process and rules for making a decision – *i.e.* to reject or to accept an analytical method – with respect to its ability to achieve reliable results.

Aim: An innovative universal strategy using Total Error is thus proposed to decide about methods' validity that controls the risk of accepting an unsuitable assay together with the ability to predict the reliability of future results. Several examples of applications of this validation methodology to various types of assays [LC-MS, ELISA, Bio-Assays] will be presented.

Method: Total error is the simultaneous combination of systematic (bias) and random (imprecision) error of analytical methods. Using validation standards both types of error are combined through the use of a prediction interval (β-expectation tolerance interval). Finally, an accuracy profile is built by connecting, on one hand all the upper tolerance limits, and on the other hand all the lower tolerance limits. This profile combined with pre-specified acceptance limits (*e.g.* 30% of bioanalysis) allows to evaluate the validity of any quantitative analytical method.

Results: The accuracy profile determines a region of results where a defined proportion of future measurements will be included inside the acceptance limits. If the analyst is willing to take, for example, a risk of 5%, this approach can give the laboratory as well as the regulatory authorities the guarantee that 95 times out of 100 the future measurements of unknown samples using the validated method will be included within the acceptance limits assessed according to the requirements. The accuracy profile is used to select the most appropriate standard curve, to estimate the limit(s) of quantification, to evaluate a potential matrix effect and, nonetheless provide estimates of measurement uncertainty.

Conclusions: This validation methodology approach allows the analysts as well as the regulatory bodies to know the risk to obtain future results out of the specified acceptance limit. Validation criteria such as the selection of the adequate standard curve and definition of the lower and upper limits of quantitation are straightforward and fit perfectly to their respective definition. Using the proposed approach, each analyst can predict the quality of the results that he will provide and thus earn confidence in the subsequent critical decisions made.

Keywords: validation, total error, accuracy profile, risk

O76. Use of accuracy profile for the validation of a gas chromatography-negative chemical ionization tandem mass spectrometry method: quantification of ethyl glucuronide in hair

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Introduction: Ethylglucuronide (EtG) is a direct and specific metabolite of ethanol. Its determination in hair is of increasing interest for detecting and monitoring alcohol abuse. The quantification of EtG in hair requires analytical methods showing highest sensitivity and specificity. We present a fully validated method based on gas chromatography-negative chemical ionization tandem mass spectrometry (GC-NCI-MS/MS). The method was validated using French Society of Pharmaceutical Sciences and Techniques (SFSTP) guidelines which are based on the determination of the total measurement error and accuracy profiles.

Methods: Washed and powdered hair is extracted in water using an ultrasonic incubation. After purification by Oasis MAX solid phase extraction, the derivatized EtG is detected and quantified by GC-NCI-MS/MS method in the selected reaction monitoring mode. The transitions m/z 347 / 163 and m/z 347 / 119 were used for the quantification and identification of EtG. Four quality controls (QC) prepared with hair samples taken post mortem from 2 subjects with a known history of alcoholism were used. A proficiency test with 7 participating laboratories was first run to validate the EtG concentration of each QC sample. Considering the results of this test, these samples were then used as internal controls for validation of the method.

Results: The mean EtG concentrations measured in the 4 QC were 259.4, 130.4, 40.8, and 8.4 pg/mg hair. Method validation has shown linearity between 8.4 and 259.4 pg/mg hair ($r2 > 0.999$). The lower limit of quantification was set up at 8.4 pg/mg. Repeatability and intermediate precision were found less than 13.2% for all concentrations tested.

Conclusion: The method proved to be suitable for routine analysis of EtG in hair. GC-NCI-MS/MS method was then successfully applied to the analysis of EtG in hair samples collected from different alcohol consumers.

Keywords: ethyl glucuronide, hair, accuracy profile, alcohol markers

O77. Screening for pharmaco/toxicologically relevant compounds in biosamples using high mass resolution. A metabolomic approach

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Introduction: The screening for pharmaco/toxicologically relevant compounds (PTRC) in biosamples has benefited a lot from MS techniques. The library search approach has enabled the development of effective identification methods based on comparison of unknown and reference spectra. However, a downside of this approach is the limited number of reference mass spectra, particularly in the case of LC-MS where in-house/commercial databases typically include not more than one thousand compounds. High mass resolution (HRMS) enables the identification of a molecular formula (MF) through the accurate measurement of mass and isotopic pattern. However, the identification of an unknown compound starting from MF requires additional tools: (a) a database associating MFs to compound names, and (b) a way to discriminate between compounds with identical MF.

Aims: To evaluate the ability of a novel metabolomic approach to reduce the list of candidates with identical MF.

Methods: Urine/blood/hair samples collected from real positive cases were submitted to a screening procedure using ESI-MSTOF (positive ion mode) combined with either capillary electrophoresis or reversed phase LC. Detected peaks were searched against a PTRC database (*ca.* 50.500 compounds and phase I and II metabolites) consisting of a subset of PubChem Compounds. In order to discriminate between compounds with identical MF a novel metabolomic approach based on the mass defect filtering technique was evaluated. Starting from the mass of the unknown compound, defects/increments corresponding to fixed biotransformations (*e.g.* demethylation, hydroxylation, glucuronidation, etc.) were calculated and the corresponding mass chromatograms were extracted from the total ion current (TIC) in order to search for metabolite peaks. For each candidate in the retrieved list, the number of different functional groups in the molecule (N,O,S-methyls, hydroxyls, acetyls, etc.) was automatedly calculated using E-Dragon software (Talete srl, Milan, Italy). Then, the presence of metabolites in the TIC was matched with functional groups data in order to exclude candidates whose structure was not compatible with observed biotransformations (*e.g.* loss of methyl from a structure not bearing methyls, glucuronidation on a structure not bearing any site susceptible to conjugation).

Results: The procedure was tested on 90 compounds detected in real positive samples, including drugs of abuse (*e.g.* cocaine, opiates, MDMA), anticonvulsants (*e.g.* gabapentin, carbamazepine), benzodiazepines (*e.g.* flurazepam), antidepressants (*e.g.* citalopram, trazodone, fluoxetine, amitriptyline, venlafaxine), phenothiazines (*e.g.* chlorpromazine, promazine, pericyazine), antipsychotics (*e.g.* amisulpride), antihistamines (*e.g.* oxomemazine), acetyl-cholinesterase inhibitors (*e.g.* rivastigmine), histamine H2-receptor antagonists (*e.g.* ranitidine), and their phase I metabolites. The mean list length (MLL) of candidates retrieved from the database was 6.81±5.12 (range 1-28) before the application of the metabolomic approach and was reduced to 4.06±3.02 (range 1-13) after.

Conclusion: HRMS allows a much broader search for PTRC than other screening approaches. The metabolomic approach significantly reduces (*ca.* 40%) the list of candidate isobaric compounds.

Keywords: general unknown screening, high resolution mass spectrometry, mass defect filtering

O78. Searching for biomarkers of disease through an untargeted metabonomic approach

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Introduction: As pioneered by Jeremy Nicholson at Imperial College London, 1 metabonomics (or metabolomics) is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification". The strategy of untargeted analysis (or metabolic profiling) is employed to detect a wide range of biochemical classes in different human biofluids (serum, urine, breath...) and obtain a large picture of metabolites; data from different platforms/methodologies can be combined to obtain as large a picture as possible. This approach has been used in toxicology, disease diagnosis and other biomedical fields.

Aim: The purpose of our work was to investigate on the potentials of full scan high resolution mass spectrometry (HRMS) with an Orbitrap instrument for the metabolic profiling of human serum and the detection of biomarkers indicative of disease. We thus compared the untargeted metabolic profiles obtained by liquid chromatography (LC)-HRMS in serum in a case-control study employing a small set (20 cases and 20 controls) of samples obtained from a intrauterine growth restriction (IUGR) investigation.

Methods: Serum samples were deproteinised by mixing with methanol at room temperature and centrifugation. Supernatants were lyophilized and reconstituted in water prior to analysis. LC-HRMS analysis were performed using an Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) hyphenated to a Surveyor Plus LC. Samples were injected onto a 1.0 × 150 mm Luna C18 column (Phenomenex, Torrance, CA, USA). The samples were eluted using a flow rate of 0.1 mL/min using chromatographic gradient of two mobile phases (water, 0.1% formic acid and methanol, 0.1% formic acid). An electrospray source was used, working in both positive ion and negative ion mode. Spectra were collected in full scan mode at a mass resolution of approximately 30,000 (full width half maximum). Data were acquired over the m/z range 50–1,000. The measurements were performed in triplicate, to account for any analytical variability. The raw data from the analysis were transformed to peak tables using XCMS software (http://massspec.scripps.edu/xcms/xcms.php) including statistics tools. To observe metabolic variation between the two sets of samples, we fitted a Principal Component Analysis (PCA) model on the LC-HRMS data. Significant differences between the ion abundances in the two sample groups were determined by Analysis of Variance (ANOVA).

Results: A large number of features (ionic species with specific retention times) were shown to differ between the two classes of samples. From their accurate mass measurements, elemental compositions of the difference molecules could be calculated, and chemical identification of those metabolites is now in process, by comparison with available data bases. Further validation study are required employing a larger number of samples.

Conclusions: The results obtained by an untargeted metabolomic approach in serum samples from an IUGR case control study put in evidence that high resolution mass spectrometry and bioinformatic data analysis can be efficiently employed to discover differences between a "normal" and a "pathological" status.

Reference: [1] Nicholson, J.K., Lindon, J.-C., Holmes, E. (1999). "Metabonomics": understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica.* 11: 1181-1189.

Keywords: metabolic profiles, LC-HRMS, case control study.

O79. Characterization of illicit amphetamines through 13C/12C and 15N/14N isotopic ratio measurements

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Introduction: Amphetamine and related drugs are psychostimulants which act by increasing levels of norepinephrine, serotonin and dopamine in the brain. Today, the designer drug 3,4-methylenedioxymethamphetamine (MDMA) is the most common amphetamine on illicit market and it is produced in several clandestine laboratories all over Europe.

Aims: Our aim was to discriminate the origin of different seizures through the analysis of: i) impurities due to manufacturing processes, which should be similar for sample belonging to the same batch, and ii) the measurement of natural abundance of stable isotopes 13C/12C and 15N/14N, expressed as δ13 and δ15 and supposed to depend on both chemical and physical processes applied for the synthesis and the origin of precursors used. Data obtained were elaborated through chemometric techniques (cluster analysis and PCA).

Methods: Twelve ecstasy tablets were analyzed. After dissolution of MDMA tablets in carbonate buffer and shaking with dichloromethane, the extracts were dried and reconstituted in 50 µl of methanol. Analysis were performed with GC-MS (Agilent Technologies) and with GC-C-IRMS (Thermo Electron). The δ13 and δ15 values were expressed as mean of five

consecutive injections of the same extract. Statistical analysis was performed by SPSS 16.0. The data matrix obtained from chemical profiling and isotopic ratio analysis was examined using Pearson's correlation test to eliminate the redundant variables. The principal component analysis and the hierarchical cluster analysis were used to classify all samples.

Results: Large variability in tablets composition was registered, as expected. Several amphetamine-like drugs (AMP, mAMP, MDEA, MBDB) were detected as well as various impurities (lubricants and adulterants), which turned out specific for the synthetic process used in the production. Upon chemometric elaboration, the amphetamine tablets were properly discriminated and clustered in a dendrogram.

Conclusions: We investigated a new approach to discriminate the origin of different seizures of illicit amphetamine-like drugs. Chemical variables including impurity profiling and natural abundance of stable carbon and nitrogen isotopes were used. The 15N/14N ratio proved particularly significant, as a specific parameter. The evidence of nitrogen isotopic fractionation can be explained in terms of differences in synthetic pathways, operating conditions and precursors used for the synthesis. Further elaboration of results is progressively updated, as long as new samples are collected for profiling, in order to confirm the usefulness of GC-C-IRMS analysis in the classification of MDMA tablets.

Keywords: amphetamines, isotopic ratio, chemometry

O80. Transfer of heroin profiling method from conventional GC-MS to Fast-GC-MS and UHPLC-MS/MS

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Introduction: Classical databases used to profile heroin samples are actually feeded with GC-MS data. Despite its ability to obtain suitable results for the classification of heroin samples and the establishment of chemical similarity [1], this chromatographic technique could be time-consuming and not available in all laboratories.

Aim: The aims of the study were, firstly, to optimize the separation and detection of the relevant components for classification of heroin samples. Secondly, to shorten the GC-MS analysis time thanks to geometrical transfer (fast-GC). Thirdly, to use alternative fast chromatographic techniques, such as UHPLC, for the analysis of heroin samples after optimisation with dedicated modelisation software. In this work, a particular attention was paid to the development of generic chemometric strategies to convert data afforded by any other analytical technique to compare with the results obtained with the classical GC-MS.

Method: The selected variables are the normalized area of specific fragments of six alkaloids co-extracted from opium with heroin. These alkaloids are meconin, acetylcodeine, acetylthebaol, 6-monoacetylmorphine, papaverin and noscapin. Linear and non-linear transformation methods were evaluated for comparison purpose.

Results: Using geometrical transfer rules and chemometric strategies, the original GC-MS method of 30 min was shortened to less than 5 min in fast-GC-MS. While correlation between the variables coming from fast-GC and GC was around 98%, a conversion factor was mandatory to implement the original database with fast-GC-MS values. Concerning UHPLC, electrospray ionisation was found to be adapted for the detection of the above mentioned compounds in less than 4 min. and can also be provide results which can be added to the original database after data correction.

Conclusions: Using those strategies, analysis times were reduced down to 5 min and the time devoted to derivatization (mandatory for GC-MS and fast-GC) could be avoided with the help of liquid based separation techniques. The time needed to establish the chemical similarity between heroin samples is now shorter and give the opportunity to the forensic scientist to play a deeper role with the investigators.

References: [1] Guéniat O. and Esseiva P., Heroin and cocain profiling, 1st Edition, 2005, Lausanne.

O81. Dosis facit venenum?

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Introduction: The fundamental statement of Paracelsus on the nature of poisons is undoubtedly a milestone in the history of toxicology, and as such a daily accompanyon of toxicological thinking and practice.

Nevertheless, its origin, its original aim and its fate through more than three centuries show some peculiarities, which are much less generally known and deserve a closer look and contemplation:

-The most cited version in Latin is not the original text, and it represents a deliberate deviation of the translator.

-The scientific reasoning of the characterisation of poisons appears as a peculiarity in the philosophical thinking of Paracelsus and is exclusively mentioned in a defensive text, so that the general aim of this statement remains somewhat unclear.

-Unclear remains as well, whether this idea was an individual prophecy or was shared among physicians / scientists of those days.

-Why was his enlightenment neither adopted and cited through the following centuries, nor by the founders of scientific toxicology around and after 1800?

Aim: From this scope, the history and fate of the remarkable sentence of the Swiss physician, philosopher and scientist Aureolus Theophrastus Bombastus von Hohenheim is outlined.

Result: Paracelsus deserves the fame, that his enlighting philosophy of drugs and poisons in the 16th century was far in advance compared with the conscience of this time, but the unknown translator of the statement and the responsible publishers deserve also a part of our admiration.

Conclusion: We encounter the fact, that this fundamental knowledge of the relativity of poisons, poison action and toxicity has even not yet become part of the general subconscious thinking and arguing of our public today.

O82. Determination of four pyrethroids pesticides in blood by solid-phase extraction and negative-ion chemical ionization gas chromatography/tandem mass spectrometry

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Introduction: Pyrethroids pesticides are widely used on crops like cotton, fruits and lettuce. Comparing to OP pesticides, pyrethroids pesticides are much more "sticky" and will rapidly absorb to suspended particulates; they can be expected to persist longer in the environment and typically much more toxic. To assist the police for investigation purposes, sensitive and reliable methods of analysis of pyrethroids pesticides are necessary in forensic laboratories.

Aim: The purpose of our work was to develop and establish the validity of a routinely applicable method that allows quantification of four common-used pyrethroids pesticides, which are fenpropathrin, lambda-cyhalathrin, cypermethrin and deltamethrin.

Methods: A quantitative method consisting of solid-phase extraction (SPE) followed by negative-ion chemical ionization gas chromatography/tandem

mass spectrometry (NICI-GC/MS/MS) analysis was developed for the identification and quantitation of four commonly-used pyrethroid pesticides. 1 ml of whole blood with the four pyrethroids pesticides were diluted with 7 ml distilled water and then extracted by C18 SPE directly. The eluted solution was concentrated and analyzed by NICI-GC/MS/MS. The following quantitation transitions wer used: 181 for fenpropaathrin, 197 for lambda-cyhalathrin, 181 for cypermethrin and 253 for deltamethrin.

Results: The best GC separation was achieved using an optimized temperature programming. For the whole blood samples, an SPE procedure to clean up the matrices was carried out prior to NICI-GC/MS/MS analysis. Under the optimum conditions, the limits of quantification of the four pyrethroid pesticides ranged from 0.02 to 0.1 mg kg(-1) with relative standard deviations <20%, and the mean recoveries ranged from 71.2 to 105.3%. The proposed method has been successfully applied to the determination of pyrethroids in two cases with satisfactory results.

Conclusion: Coupled with SPE, NICI and MS/MS, GC analysis offers a better selectivity and sensitivity than exist ones when analyzing pyrethroids pesticides.

Keywords: pyrethroids pesticides, GC/NICI-MS/MS, whole blood, SPE

O83. Impact of lead exposure on serum paraoxonase 1 (pon1) activity and genotype in occupationally- exposed Egyptian workers

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Rationale and background: The continued occurrence of occupational lead over-exposure and lead poisoning in Egypt remains a serious problem despite awareness of its adverse health effects. Meanwhile, an association between lead exposure and serum cholesterol and lipoprotein levels was found in workers of battery and recycling factories. A hypothesis was originally based on the finding that purified paraoxonase-1 (PON1) was highly effective in preventing lipid peroxidation of LDL. Lead and several other metal ions were claimed to inhibit PON1 activity *in vitro* thus producing atherosclerosis.

Objectives: The aim of the present study was to study whether lead exposure has any effects on serum PON1 activity and lipid profile levels

Subjects and methods: This study was carried out on 90 workers (90 males) in a lead-acid battery manufactory. Full medical history and clinical examination were performed with special emphasis on blood pressure measurement. Blood samples were withdrawn from every selected worker for estimation of blood lead levels, the separated serum was used for estimating lipid profile as well as and liver and renal function tests in addition to blood glucose levels and PON1 activity assay. Buffy coat isolated from EDTA-treated blood was used for genomic DNA preparation for genotyping. According to the obtained blood lead level, subjects are classified into 3 groups: group I with blood lead level <40 µg/dl, group II with a level ranged between 40 and 60 µg/dl and group III with a level above 60 µg/dl.

Results: No statistically significant difference was found in systolic and diastolic blood pressure between the different groups. Only total cholesterol and LDL-C levels were statistically significantly different among the three groups, with the highest levels in the highest-exposure group. Moreover, the average paraoxonase activity of the high-exposure group was lower than the low-exposure group however, it didn't reach statistical significance. Simple linear correlation analysis revealed that, blood lead was positively correlated with total cholesterol and LDL C (P=0.04). In addition, a highly significant negative correlation was found between paraoxonase and both age of the workers and their duration of exposure (P=0.002, 0.005, respectively). A significant negative correlation was found between paraoxonase and blood lead in all groups studied as one. Blood lead was found to be an independent factor affecting serum PON1 activity P= 0.05. The present study failed to find that the subjects who are homozygous for the R allele are more susceptible to lead toxicity than are subjects of other genotypes.

Conclusion and recommendations: Increasing the duration of occupational lead exposure is associated with decreased serum PON1 activity, which has a protective role against the development of atherosclerosis.

O84. Evaluation method for linking drug seizures using stable carbon and nitrogen isotopic compositions: a complementary study with impurity profiling

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Introduction: Drug profiling, extraction of physical and/or chemical profiles from abused drug samples, is useful for inferring and characterizing links between samples originating from the same and different seizures, and supports drug crime investigations. We conducted impurity-profiling analysis, one of the major methods of drug profiling, on methamphetamine (MA) seized by Japanese police over more than 15 years using capillary gas chromatography. This analysis has been very effective for determining sample similarities. However, when the samples contain very few impurities, evaluation becomes very difficult. Besides, forensic analysts have to be very careful in concluding that 2 samples are the same, especially when the samples belong to different cases. Conducting other analytical procedures based on different principle and adding information on MA profiling would be preferable.

Aim: The purpose of our work is to develop an evaluation method for linking MA seizures using stable carbon and nitrogen isotopic compositions concurrently with gas chromatographic impurity profiling.

Methods: We analyzed both impurity profiles and carbon and nitrogen stable-isotopic compositions of several sets of seized MA whose investigative information indicated that each set had the same origin. The size of the cluster in the isotopic-composition graph was determined with pooled standard deviations (SDs), the pooled estimates of measurement uncertainty. Linkages between different cases were evaluated with the stable-isotopic compositions and the determined pooled SDs.

Results: The impurity profile of each set of seizures was quite similar and hierarchical cluster analysis showed sample classification that was relatively consistent with the investigative information. Stable carbon and nitrogen isotopic compositions of the MA seizures varied between -29.40 and -24.90‰ (δ13C) and -2.29 and 5.94‰ (δ15N), respectively. In the δ13C–δ15N graph, MA seizures were classified into 7 groups, probably reflecting different origins. The size of the cluster in the isotopic-composition graph was determined by pooled SDs (sp). The sizes of the clusters were less than 6sp and the linkages between the MA seizures from the isotopic compositions were consistent with the impurity profiling and investigative information.

Conclusion: A criterion of the stable-isotopic composition for linking MA seizures was estimated with pooled SDs and used for evaluating the relationships between different cases. The results showed that complementary use of stable-isotopic compositions with impurity profiling provides useful information for evaluating the links between seizures.

Keywords: stable isotope ratio mass spectrometry, gas chromatography, drug analysis, impurity profiling, methamphetamine seizure, pooled standard deviation, amphetamine-type stimulants (ATS)

O85. Sample preparation in systematic toxicological analysis

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Introduction: The TIAFT Committee on Systematic Toxicological Analysis is preparing Recommendations for each stage of the analytical strategy used in identifying potentially toxic compounds and their metabolites in biological samples. After addressing the basic issue of sampling (recommendations for which will be published in the next TIAFT-Bulletin), the subsequent step of sample preparation should then be considered. During the process of systematic toxicological analysis (STA) some toxic compounds can be detected directly after digestion of the sample matrix (*e.g.* metals), or they can easily be separated from less volatile matrix components (*e.g.* gases and volatile compounds). However, less volatile drugs and poisons such as drugs-of-abuse, pharmaceuticals, herbicides and pesticides form a very important toxicological group, and their isolation requires more complex extraction procedures.

Aim: This presentation aims to promote discussion about recommendations for sample preparation in systematic toxicological analysis.

Methods: The presentation is based on a review of the relevant literature with a focus on extraction procedures for screening and/or STA.

Results: Generally, the physico-chemical properties of toxicologically relevant compounds differ greatly; in the case of STA, they are unknown - as is the composition of the specimen. Therefore, non-selective extraction procedures covering a wide variety of possible target compounds are needed. For liquid-liquid extraction, mixtures of solvents are often used. For solid-phase extraction, so-called "mixed-mode sorbents" are frequently applied. A selective increase of the concentration of target compounds relative to co-extracted matrix compounds (enrichment) and a selective separation from interferences (purification) is not possible in STA, because the extraction procedure can not be optimized for one particular compound and/or specimen. The resulting co-extraction of a large number of interferences from the sample matrix demands powerful analytical procedures to ensure accurate separation, detection and identification of the unknown compounds. Moreover, in both extraction techniques (liquid-liquid and solid-phase extraction), pretreatment of the sample is an important precondition for a successful extraction.

Conclusions: The isolation of the compound/s of interest from the biological matrix is essential for their successful detection and identification. Because of the large number of toxicologically relevant compounds and the different specimens which have to be analyzed, one particular standard extraction procedure for STA does not exist. Therefore, the Committee on STA´s Recommendations for sample preparation focus on the basic requirements for the applied procedures, illustrated by practical examples.

Keywords: systematic toxicological analysis, STA, sample preparation, recommendations

O86. Monitoring of herbal mixtures potentially containing cannabinoid designer drugs as psychoactive adulterants

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Introduction: Herbal mixtures with potentially bioactive ingredients were available in almost all European countries since 2006 and are widely used as a substitute for cannabis, although merchandised as 'herbal incense'. After gaining a high degree of popularity by mid of 2008, big quantities of these drugs were sold, particularly in Germany, Austria, France and Great Britain. In December 2008, German labs identified different synthetic cannabinoids in the mixtures not declared as ingredients: A non-classical cannabinoid CP 47,497 (a potent CB1 and CB2 agonist), the C8 homologue of this compound, and two cannabimimetic aminoalkylindoles called JWH 018 and JWH 073. This kind of products attracts especially young people and first time consumers since they are available without age restriction and marketed as "legal" and "natural". Habitual cannabis users also use these drugs particularly when facing abstinence controls or the risk of getting caught driving under the influence of drugs, since these compounds cannot be detected by immunoassay based tests so far. Despite prohibition in some countries by national legislation (Austria, Germany, France), these products are still easily available *e.g.* via Internet shops. So far not much is known about metabolism and toxicology of these compounds. There is the risk of full CB receptor agonists leading to life threatening conditions after overdosing (unlike Δ9 THC which acts as a partial agonist). Furthermore, it seems to become manifest that tolerance to these synthetic cannabinoids develops quite quickly, with the likely consequence of a relatively high addictive potential.

Aim: The purpose of the presented work is a continuous monitoring of the 'incense' market, in combination with performance of metabolic and toxicological studies for the identified designer cannabinoids.

Methods: For product monitoring, ethanolic extracts of the herbal mixtures (0.1 g in 1 mL solvent) were analysed by GC-EI-MS in scan mode. After elucidation of the cannabinoid structures by various MS techniques, preparative HPLC and NMR, the purified compounds were enzymatically metabolised by using human liver microsome preparations, in order to look for potential biomarkers.

Results: After analysis of more than forty different herbal mixtures which were/are available via Internet or headshops, it was found that 21 of them contained synthetic cannabinoids (CP 47,497, its C8 homologue, JWH 018 or JWH 073); while in 18 of them the presence of synthetic adulterants could not be proved. Three of the products contained natural constituents, such as harmine, harmaline, myristicine or natural cannabinoids.

Various oxidative metabolites have been detected, yet the specific metabolic pathways of the designer cannabinoids and the evaluation in terms of suitability as consume markers are still under study.

Conclusions: The convenient possibility of purchase via Internet and the lack of cross reactivity to conventional drug screening procedures make these herbal mixtures very attractive for certain groups of consumers. Detection and identification of psychoactive drugs added to these mixtures, together with looking for adequate consume markers in blood and urine involves an ongoing challenge for toxicologists.

POSTER PRESENTATIONS

Post mortem toxicology

P001. Toxicological findings in two planned complex suicide cases: ingestion of petroleum distillates and subsequent hanging

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Introduction: Complex suicides referring to suicide by a combination of more than one method. The use of multiple methods of self-destruction during a single suicide episode is a rare occurrence and toxic compounds are used in few of these reports. A careful evaluation of all elements, including examination of the scene and anatomopathological and toxicological findings, can reconstruct the lethal chain of events and elucidate the cause, manner, and mechanism of death.

Aim: This paper describes two fatal cases of planned complex suicides in two male individuals, of 86 and 51 years old, involving ingestion of petroleum distillates and hanging. Remarkable internal findings during autopsy of both

cases were the intense odor of petroleum distillates that alerted authorities to the suspicion of ingestion.

Methods: The initial toxicological screening and quantitation of these compounds were performed by means of gas chromatography with flame ionization detector (GC-FID) and confirmation was performed using gas chromatography-mass spectrometry (GC-MS) total ion chromatogram (TIC) mode after liquid-liquid extraction of biological samples.

Results: Case 1: Diesel fuel No. 2 concentrations were: Heart blood < 5 mg/L and gastric content 18.168 mg/L (total amount 6.356 mg). Therapeutic concentrations of citalopram were also found in blood. Case 2: Xylene (mixture of isomers) concentrations were: Heart blood 0.3 mg/L and gastric content 0.1 mg/L (total amount: 0.006 mg). Ethanol (1.12 g/L) and therapeutic concentrations of nordiazepam, oxcarbazepine, ibuprofen, and metamizol were also found in blood.

Conclusions: The medical examiners reported in both cases the cause of death as hanging and based upon the examination of the scene, the anatomopathological, and toxicological data the manner of death was determined to be planned complex suicide. We would like to alert toxicologists of the importance of testing for petroleum distillates, when there is a suspicion of ingestion of these products due to the odor observed at scene of death and/or during autopsy. The results of these toxicological investigations, can help to discriminate the manner of death and the medicolegal interpretation.

Keywords: petroleum distillates, complex suicides, hanging, analysis, toxicology

P002. A fatality attributed to GBL overdose

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Introduction: Gamma-hydroxybutyrate (GHB) is produced endogenously as a by product of gamma-aminobutyric acid (GABA) metabolism. It has become a popular recreational drug, which is associated with significant morbidity and mortality. It is therefore a class C drug under the Misuse of Drugs Act (1971). However, its prodrugs gammabutyrolactone (GBL) and 1,4-butanediol (1,4-BD), which are widely used in the chemical industry, remain legally available in the UK despite having similar clinical effects. There have been suggestions in the medical and general press that there has been a shift amongst GHB users to GBL and other precursors such as 1,4-BD, due to the disparity in the current UK legislation.

Aim: Despite the evolving trend towards GBL use, to date there are no publications attributing fatalities directly to GBL. Due to the rapid in-vivo conversion of GBL and 1,4-BD to GHB in equimolar ratios, there are challenges associated with distinguishing between the use of these compounds through analysis of biological specimens alone. Methodologies involving conversion of GHB to GBL and measurement of 'total' GBL are common. We present a case study of a fatality attributed to GBL, confirmed by analysis of the liquid consumed.

Methods: Analysis of the biological samples for 'total' GBL was performed after conversion of any GHB present to GBL. A Shimadzu GC-MS-QP2010 with a AOC-20i autosampler and HP-5MS (30 m × 0.25 mm, 0.5 µm) column was used. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injection volume was 1.0 µL in splitless mode. The initial column temperature was set at 80 °C and held for 4 mins. It was then ramped by 25 °C/min up to 125 °C. The total run time was 6 mins. Positive Electron Impact Ionisation (EI) mode was used and data were collected using single ion monitoring (SIM). GBL and GBL-d6 were quantified monitoring m/z: 86 and 92 and their retention times were 4.64 and 4.68 minutes, respectively. The case liquid was analysed by diamond ATR infra-red spectroscopy (IR). GHB has a strong band at ~3500 cm^{-1} which is not present in GBL. There are also marked differences in the fingerprint region allowing differentiation.

Results: The deceased was a 25 year old male who was feared to have overdosed on GBL after failing to use a measuring pipette for dosing. Total GBL was detected in the preserved femoral blood at a concentration of 282 mg/L. Fatality has been associated with concentrations exceeding 280 mg/L. Ephedrine was present at <0.05 mg/L. No alcohol or other common drugs were detected. The IR liquid screen was consistent with GBL. The pathologist attributed the cause of death to respiratory failure secondary to GBL toxicity.

Conclusions: GBL use may be more common than previously thought. There is a need for further work to determine whether GBL is associated with morbidity and mortality similar to GHB. Continued monitoring of the use of GBL, 1,4-BD and other GHB prodrugs and precursors will be invaluable to determine how the market adapts following the pending change in legal status of GBL and 1,4-BD to Class C controlled drugs.

Keywords: gamma-hydroxybutyrate (GHB), gammabutyrolactone (GBL), 1,4-butanediol(1,4-BD)

P003. The *post mortem* relationship between beta-hydroxybutyrate, acetone and ethanol in ketoacidosis

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Introduction: A reduced blood pH (ketoacidosis) from the production of b-oxidative ketone bodies can be a result of various medical reasons, particularly alcohol abuse and diabetes. Such conditions feature in many fatalities and analytical evidence can be used to support a pathological diagnosis, or provide a possible cause of death in the absence of other pathologically significant findings. Current testing may involve analysis of the common ketone bodies, beta-hydroxybutyrate (BHB) and acetone, this is usually coupled with ethanol and glucose determination for suspected alcoholic ketoacidosis (AKA) and diabetic ketoacidosis (DKA), respectively. It is generally believed and published that a) ethanol is low or absent (<10 mg/dL) in AKA, b) the absence of acetone does not necessarily preclude ketoacidosis (BHB still needs to be measured), c) BHB concentrations in blood below 500 mmol/L (52 mg/L) are normal and greater than 2500 mmol/L (258 mg/L) are pathologically significant.

Aims: In order to re-examine existing beliefs in the analytical investigation of ketoacidosis, the relationship between common ketone bodies (BHB and acetone) and ethanol was studied. Many publications involve enzymatic measurement of BHB, this paper utilises previously developed GC-MS methodology for specificity to determine if published BHB concentration ranges still apply.

Methods: The presence of acetone and measurement of BHB and ethanol was collated from over 350 fatalities grouped into a) alcoholics, b) diabetics, c) alcoholic + diabetic, d) speculative and e) controls (alternative cause of death). The concentration of BHB and ethanol in *post mortem* blood, urine and vitreous humour was determined using GC-MS and headspace GC-FID, respectively. The presence of acetone was detected using headspace GC-FID.

Results: All control cases had blood BHB concentrations below 50 mg/L. In alcoholics, 38% had a low blood BHB concentration (<50 mg/L) compared to 34% with a high BHB concentration (>250 mg/L), the majority (55%) of urine BHB concentrations were >250 mg/L. In diabetics, most (49%) had a high blood BHB concentration (>250 mg/L) compared to 31% with a low BHB concentration (<50 mg/L), the majority (70%) of urine BHB concentrations were >250 mg/L. In alcoholic diabetics, 61% had high blood and 69% had urine BHB concentrations (> 250 mg/L). 30% of speculative cases were found to have pathologically significant BHB concentrations (> 250 mg/L). Across all groups, only one case involved a blood BHB concentration >250 mg/L but urine BHB <250 mg/L. 19% of all cases involved urine BHB concentrations >250 mg/L but blood BHB <250 mg/L. Vitreous humour BHB concentrations were comparable to blood with only

one case involving a BHB concentration <250 mg/L but high vitreous concentration >250 mg/L. Acetone was detected in all cases where blood BHB was >250 mg/L. In alcoholic and alcoholic diabetic groups, ethanol was absent in 59% of high BHB cases (>250 mg/L) and absent in 55% of low BHB cases (<50 mg/L).

Conclusions: It is necessary to re-evaluate current beliefs. The study data showed ethanol is not always low (<10 mg/dL) or absent in cases of AKA. Also, the absence of acetone does not preclude a pathologically significant ketoacidosis (high BHB), therefore acetone can be used as an initial marker. For blood and urine BHB concentrations the following interpretative ranges can be used (in mg/L); normal (<50 mg/L), raised (51-249 mg/L), high and pathologically significant (>250 mg/L). Initial data suggest vitreous humour BHB could be a useful alternative in the absence of blood (same interpretative ranges may also apply).

Keywords: beta-hydoxybutyrate, acetone, ethanol, ketoacidosis

P004. A fatally mistaken fruit juice drink: an unordinary way of cocaine intoxication

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Introduction: According to data released by the European Monitoring Centre for Drugs and Drug Addiction, an increase in cocaine consumption has been recorded in Spain. In the last few years, cocaine has emerged as one of the issues that arises higher concern in our society. Furthermore, due to its geographical position Spain is used by organized drug traffickers as the drug port of entrance in the European Union.

Aim: The aim of the present paper is to report a case of a fatal intoxication with cocaine ingested by mistake. A 38 years-old male who suffered of leukaemia was recommended to drink a juice of a tropical fruit called Noni in order to reduce side effects of cancer treatment. In our country, this beverage was sold only by the Internet by a Mexican distributor. He received at home three bottles of the fruit juice. He opened one of them and drunk a little quantity. Suddenly, he felt very bad, falling death on the floor. Although some kind of allergic response was alleged as the cause of the death, the coroner sent us blood, urine and vitreous humour along with the bottles of Noni.

Methods: Toxicological analyses were performed in all the samples received, following our laboratory standard procedures. Ethanol was analysed by means of headspace GC-FID. Screening of drugs of abuse was performed by means of homogeneous enzyme immunoassay CEDIA, and then SPE (Bond-Elut, certified) was performed in all the samples. The presence of cocaine and its metabolites were confirmed and quantitated by GC-MS, previous derivatization with BSTFA.

Results: Ethanol, cocaine, methylecgonine, benzoylecgonine and ethylbenzoylecgonine were detected in two of the three bottles of fruit juice received. No ethanol was detected in blood. Cocaine, methylecgonine and benzoylecgonine were detected in blood, urine an vitreous humour, as it is shown in the table below (mg/L):

	Blood	Urine	Vitreous Humour
Cocaine	2.27	15.51	4.55
Benzoylecgonine	5.31	25.31	3.85
Methylecgonine	14.74	14.06	4.47

Conclusion: Cause of death was reported as fatal intoxication by cocaine. It is the first time, to our knowledge, that it is reported cocaine concealed in this kind of tropical fruit juice. As it is widely known, buying medicines, drinks and food in Internet presents a potential danger to Public Health. Cases as the one reported here confirms this statement due to the fact that a diversion in the distribution net of drug traffickers leaded to fatal consequences in an innocent person.

Keywords: cocaine, tropical fruit juice, drug trafficking, death

P005. It is always the manner of death what its first looks like?

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Introduction: According to our annual statistics, our laboratory receives an average of 5000 cases per year. Of these cases, manner of death if first attributed by the coroner to an accident in 450 cases. Road accidents represent a 65% of them. Coroners frequently ask for general unknown analysis in order to discard or confirm the possible contribution of a toxic compound to death. Sometimes, first coroner's conclusions change after the toxicological analyses are reported.

Aim: The aim of the present paper is to report two cases which were first classified as accidents. Case 1: A 40 years-old male who was found dead on the beach. Case 2: A 29 years-old male who fainted while driving his car and had a road accident.

Methods: General unknown toxicological analyses were performed in all the samples received, following our laboratory standard procedures. Ethanol was analysed by means of headspace GC-FID. The presence of cocaine and its metabolites were confirmed and quantitated by GC-MS, previous derivatization with BSTFA. MDMA and MDA were confirmed and quantitated by GC-MS, previous derivatization with heptafluorobutyric anhydride.

Results: MDMA, MDA and cocaine were detected in both cases. Blood, urine and vitreous humour were analysed. In case 2 a hair shaft of 3.5 cm was also received. In case 1 BAC was 1.67 g/L. No ethanol was detected in case 2. Obtained results are shown in the table below:

	Case 1			Case 2			
	Blood	Urine	Vitreous Humour	Blood	Urine	Vitreous Humour	Hair
Cocaine	0.24	2.08	<LOD	0.16	18.40	<LOD	29.32
Benzoylecgonine	0.96	247.04	<LOQ	2.19	221.47	1.6	17.90
Ethylbenzoylecgonine	0.44	5.31	<LOD	<LOD	6.96	<LOD	0.93
MDMA	0.17	3.05	0.08	3.38	43.70	1.78	6.53
MDA	<LOQ	0.06	<LOD	0.04	1.45	0.06	0.22

Conclusions: Once the toxicological analyses were concluded, manner of death was reported as drug intoxication in both cases, in which the decedents suffered the sinergic action of cocaine and MDMA. Although blood cocaine concentrations are not considered by the majority of authors high enough to produce death, MDMA blood concentrations could justify it due to the fact that most of the published cases of toxicity or fatality with have reported concentrations between 0.5-10 mg/L. This is the first time that in our laboratory we found a MDMA and cocaine intoxication in a road accident. Hair analysis in Case 2 showed chronic consumption of cocaine and MDMA 3.5 months previously to death.

Keywords: cocaine, MDMA, combined consumption, death

P006. Evaluation of COZART DDS in samples of *post mortem* blood

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Introduction: The rising number of accidents and deaths related to the increased prevalence of drugs of abuse makes it necessary to develop simple and fast detection techniques. Immunoassay-based tests both for oral fluid and for urine

are being widely used in different clinical and forensic areas. However, the most commonly used matrix in forensic toxicology is blood, for which there is a reduced number of fast and reliable in situ tests. Since our previous studies suggested that Cozart® DDS oral fluid test appears to be reliable for oral fluid samples, we have decied to check the suitability of Cozart® DDS test for the detection of drugs of abuse in *post mortem* blood samples.

Aim: The aim of this study was to determine the suitability of Cozart® DDS test as a useful tool in the determination of drugs in *post mortem* blood.

Methods: The total sample includes 128 cases, which were collected in autopsies and stored at 4 ºC. From the total, 95 samples were suspected of being related to drug consumption and 33 related to other causes. Samples were analyzed both by Cozart® DDS 801 test and by GC/MS-MS. The analytes that can be identified using the immunoassay test are D9-THC, 6-monoacetylmorphine, cocaine, amphetamine and methamphetamine (cut-off values are 31 ng/ml for D9-THC, 30 ng/ml for cocaine and 50 ng/ml for 6-acetylmorphine, methamphetamine and amphetamine). Instead, the analytes detected by GC/MS-MS technique are D9-THC, cocaine, 6-acetylmorphine, amphetamine and methamphetamine (MDMA and MDEA with a cut-off value of 50 ng/mL for both analytes). Sample preparation consists in the addition of 1975 ml of oral fluid and 4 ml of phosphate buffer at pH 6 with 25 µL of cocaine-d3, 6-MAM-d3, amphetamine-d5, methamphetamine-d9 and D9-THC-d9 for a final concentration of 10 mg/mL. Sample is homogenised for 15 sec., centrifuged 10 min. at 10000 rpm and submitted to the solid phase extraction procedure (SPE). Organic phase is collected, evaporated since dryness and derivatized with 40 mL of BSFTA-TMCS at 80 ºC for 20 min or PFPA for amphetamines and methamphetamines at 50 ºC for 40 min. 2 mL of the sample are injected in a split-less GC/MS-MS. A temperature program is carried out starting at 90 ºC for 1 min followed by a ramp of 20 ºC/min since 240 ºC and a second ramp of 5 ºC/min since 300 ºC by using a column VF-5 ms 30 m × 0.25 mm. Detector used has been a Varian ion trap with electron impact ionization considering the MS-MS alternative with father ions, 386 for D9-THC-TMS, 182 for cocaine, 399 for 6-MAM-TMS, 204 for methamphetamine-PFPA and 190 for amphetamine-PFP. No studies aimed at determining quantitatively the analyte were performed, as the main interest lies in the qualitative detection.

Results: For the 128 cases analyzed by Cozart® DDS test and further examined by GC/MS-MS, cocaine shows a concordance ratio of the 89.3%, opiates a 80.6%, D9-THC 100% and negative cases a 75%.

Conclusion: Results allow us to conclude that Cozart® DDS test is a useful method for fast and preliminary screen of drugs of abuse owing to the high agreement observed with results confirmed by GC/MS-MS (equal or higher than a 75%).

Keywords: Cozart® DDS test, GC/ MS-MS, *post mortem* blood

P007. A fatal case due to venlafaxine overdose

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Introduction and **Aim**: Venlafaxine is a phenethylamine derivative widely prescribed for the treatment of depression. Venlafaxine inhibits the reuptake of serotonin and noradrenaline and the symptoms of its overdose may include central nervous system depression, cardiac arrhythmia, low or high blood pressure, seizures and coma. In treatment with antidepressants of patient with depression and other psychiatric disorders there is also increased risk of suicidal thinking and behaviour. Several lethal intoxications involving venlafaxine usually among psychotic patients have been reported in the literature. In the most cases venlafaxine was detected with other substances so its role in the death was not obvious. From the examined cases, it was concluded that venlafaxine even in very high concentrations was not adequate to lead to death, as other drugs were detected in high levels. In the present work, a fatal case of a 55 years old female due to venlafaxine overdose is presented.

Methods: EMIT, FPIA and thin layer chromatography were used for screening purposes and gas chromatography with nitrogen phosphorous detection was used for confirmation and quantitation of venlafaxine in the whole blood of the deceased and in the stomach and oesophagus content, after liquid-liquid extraction.

Results: Toxicological analysis revealed a high concentration of venlafaxine in *post mortem* whole blood (13.7 µg/ml). The drug was also present in the stomach and oesophagus content. Besides macroscopic examination of the stomach and oesophagus content, during autopsy, has shown portions of the drug. No other drugs or alcohol were detected in the biological samples of the deceased.

Conclusion: The death was attributed to overdose poisoning of venlafaxine, as no other substance was detected in biological samples and according to the death certificate. This case was the first fatal overdose intoxication due to venlafaxine reported in Greece.

Keywords: venlafaxine, GC-NPD, fatal case

P008. Fatal suicidal organophosphorous poisoning in Southern India

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Introduction: Poisoning is an important health hazard and one of the leading causes of morbidity and mortality worldwide. Widespread use of organophosphates in industrial and agricultural application is responsible for estimated 25 million poisoning worldwide. More than 90% of fatal poisonings occur in developing countries, particularly amongst agricultural workers. Intentional self-poisoning is reportedly the commonest form of poisoning in adults.

Aim: The purpose of the study is to assess the epidemiological variables of fatal suicidal organophosphorus poisoning to identify that people at risk and understand the magnitude and pattern of suicidal poisonings in this region.

Methods: This hospital based retrospective analysis was carried out in Kasturba Medical College, Manipal. The study spanned over a period of 6 years from January 2000 and December 2006. A detailed profile was made based on the hospital and autopsy records, information furnished by the police and chemical analysis reports from the Regional Forensic Science Laboratories (RFSL). All the cases where organophosphorus compounds were identified as the cause of fatal outcome and manner was deemed as suicide were included. The data was statistically analysed using spss version 11.0. Chi square (×2) test was performed to test the significance of each group. Pearson correlation was done to find a relation between duration of survival and age/ sex/ viscera analysis at the RSL. p-value < 0.05 was considered significant.

Results: Study included a total of 184 cases of fatal deliberate self-poisoning. Organophosphorus compounds (OPCs) were implicated in 70.7% cases (n=130). Males were predominantly affected (78.5%). Majority of the victims were in 3rd and 5th decade (66.8%). Mean age was 41.8 years in males and 33.6 years in females. In maximum number of cases (95.4%) cases OPCs were taken alone. Maximum cases were reported in the months of March (n=18) and May (n=18). Period of survival ranged from 0-58 days. Mean duration of survival was 4.9 days for males and 4.1 days for females. While 13.8% cases (n=18) were brought dead to hospital, more than half of the victims (51.5%) died within 4 days of hospital admission. Post mortem chemical analysis could identify the OPCs in the viscera and body fluids in ninety cases (69.2%). A statistically significant correlation was observed between duration of survival and the post mortem detection of OPCs. Duration of survival was however, independent of age and sex. Annual distribution in

relation to number of autopsies in our centre reveals an overall decline in fatal self-poisoning from OPCs during the study period.

Conclusion: The study highlights the pattern of fatal self-poisoning with organophosphorus compounds in Manipal, South India. India is predominantly an agriculture based country, and easy availability of agrochemicals facilitates fatal self-poisoning. Strict implementation and enforcement of laws, greater control in the sale and use of agrochemicals are recommended along with better health care facilities in rural India, to prevent poisoning related mortalities.

Keywords: poisoning, suicide, organophosphates, mortality, medicolegal autopsies

P009. Does alcohol increase the risk of death in house fire by inhalation of noxious gases?

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Introduction: Carbon monoxide (CO) is a well known toxic component in fire-related deaths. Hydrogen cyanide (HCN) may, however, also be of importance. Combustion of carbon-containing materials generates CO and carbon dioxide, while the burning of nitrogen-containing materials also generates HCN. In victims of house fire, post mortem blood concentrations of CO and cyanide (CN-) are commonly measured to distinguish death through hypoxia (lack of air (oxygen) through breathing exhaust fumes) from intoxication (breathing noxious gases generated from burning combustible materials). Interestingly, alcohol is reportedly associated with approximately 80% of fire-related deaths. We wanted to consider if this association had an influence upon the nature of death in a house fire due to the inhalation of noxious gases.

Aims: To investigate whether alcohol has a positive influence upon the risk of death from CO and/or CN- in house fire.

Methods: We measured carboxyhaemoglobin (COHb) concentration, as an index of CO exposure, and CN- concentration, as an index of HCN exposure, in *post mortem* blood specimens taken from victims of house fires in Northern Scotland between 1994 and 2009. COHb concentrations were measured using automated spectrophotometric co-oximetry systems (IL 482; AVL OMNIÔ), while CN- concentrations were determined by spectrophotometry.

Results: In 61 victims of house fires, the mean blood COHb concentration was 51.0% (range: 4.3 - 84.1%) and the mean blood CN- concentration was 1.04 mg/L (range: 0.03 - 6.94 mg/L). Concentrations of COHb >40% and of CN- >1.00 mg/L were considered fatal; in this cohort, 14 deaths were associated with fatal concentrations of both toxins, 28 with CO alone, 6 with CN- alone, and 13 with sub-lethal concentrations of both. Blood alcohol concentration (BAC) had been estimated in 55 of the cases; 38 had a significant BAC (defined as >80 mg/100 ml, the current drink driving limit for the United Kingdom). For both CO and CN-, a greater proportion of lethal concentrations were found in association with positive BAC.

Conclusion: This study shows that CO and HCN both contribute to house fire fatalities, alone or in combination, and that drinking alcohol to a BAC >80 mg/100 ml positively increases the risk of death through inhalation of one or both of these toxins in a fire.

Keywords: carbon monoxide, cyanide, alcohol, fire death

P010. Is death by potassium chloride intravenous injection undetectable by toxicological analyses?

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Introduction: Potassium chloride intravenous injection is reported as a means in suicide attempts or in lethal procedures for state-sanctioned capital punishment. In addition, it is also reported as an accidental cause of death for mistaken injection in hospitalized patients. Owing to its relatively high concentrations in hemolyzed blood (25-80 mmol/l) as compared to serum (about 4 mmol/l), potassium poisoning has often been considered hardly detectable in *post mortem* blood specimens.

Aim: In considerations of the results of the determination of blood potassium in a case of suicide by potassium poisoning, the meaning of the blood potassium concentration is questioned and discussed.

Case: A 41-year old man, working as a nurse at the local Intensive Care Unit, was found dead at his workplace. An acupuncture sign was observed on his left foot and a syringe retrieved close to the corpse. At the autopsy no particular signs were noted.

Methods: Biological specimens (blood, bile, and urine) were submitted to the screening procedure for drugs and poisons in use in the laboratory (blood and bile: general unknown screening by solid phase extraction and gas chromatography mass spectrometry; blood: head-space gas chromatography; urine: EMIT). Moreover, the aqueous liquid found in the syringe was found positive to potassium according to Feigl spot test and, consequently, blood potassium concentration was determined by ion selective electrode measurement (linear over the range 3.0-150 mmol/l).

Results: According to the routine screening procedure, blood was found positive for diazepam at a therapeutic level (0.21 mg/l) and urine resulted positive for benzodiazepines. Blood and urine showed no other positivities and all other samples tested negative. Potassium concentration was found to be 160.0 mmol/l in cardiac blood and 87.3 mmol/l in femoral blood (mean of three determinations in both cases). On the other hand, hemolized blood samples obtained from autopsies with no relevant toxicological findings had much lower potassium concentration, *i.e.* between 32.2 and 43.0 mmol/l (median: 38.6 mmol/l, n=6).

Conclusions: Death by potassium intravenous injection is often considered undetectable by analytical toxicology when only hemolized blood is available, and, consequently, literature is relatively scant. Our result show that potassium concentration resulted to be significantly higher in heart blood in a case of suicide by potassium chloride intravenous injection in comparison with standard values in *post mortem* blood and, therefore, the general issue of considering potassium poisoning hardly demonstrable by the toxicology needs to be questioned and thoroughly studied.

Keywords: potassium chloride, intravenous injection, suicide

P011. Case report: unusually high concentrations of 1-benzylpiperazine in a *post mortem* blood sample and vitreous humor sample

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Case Report: A 54 year old lady was found dead, lying face down on the kitchen floor in her home. She was last seen alive in the morning of the day of her death. The deceased had been complaining of feeling tired in the days prior to her death.

A *post mortem* examination was conducted and an unpreserved blood sample and a vitreous humor sample were collected for toxicological analysis.

Method: Routine toxicological analyses were performed on the samples and 1-benzylpiperazine (BZP) was confirmed by gas-chromatography-mass spectrometry after liquid-liquid extraction.

Results: Toxicological analysis of the blood and vitreous humor samples identified high concentrations of BZP. BZP was detected in the blood sample at a concentration of 8.3 milligrams per liter and in the vitreous humor at a concentration at approximately 12 milligrams per liter. Fluoxetine, norfluoxetine and codeine were also detected in the blood sample, at concentrations broadly consistent with therapeutic use, along with caffeine and nicotine. No alcohol or other common drugs of abuse were detected.

Conclusions: The cause of death was given as acute myocardial ischemia due to coronary artery atherosclerosis. The toxicological results were interpreted

with caution due to the limited published data on the toxic effects of BZP in humans, especially at the time of the findings. The results are higher than previous cases involving BZP where people have died from causes unrelated to this drug. The analysis of vitreous humor, which is largely protected from early post mortem changes, supports the high concentration of BZP detected in the unpreserved *post mortem* blood sample. Further studies need to assess whether BZP is prone to *post mortem* redistribution. BZP has been reported to have similar pharmacological effects to amphetamine and as is the case with amphetamine, it may be difficult to distinguish between the therapeutic, toxic and fatal range of this drug.

Keywords: 1-benzylpiperazine, *post mortem* samples

P012. On the utility of doxepin/nordoxepin concentration ratios in *post mortem* cases

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Introduction: Doxepin (Dox) is the 2nd most used sedating antidepressant in Germany, and is also most frequently associated in suicide and narcotic drug related deaths among all antidepressant agents.

Aim: Body fluids and tissues in 8 Dox related deaths were investigated in order to prove whether the individual concentration of Dox, the concentration sum of the parent drug and its active metabolite N-desmethyldoxepin (NDox) or the concentration ratio Dox/NDox valuably contribute to making a cause of death determination.

Methods: Dox and NDox concentrations were determined by means of an LC-MS/MS method. Dox/NDox ratios were analyzed with regard to autopsy findings, scene observations and information on the past social and medical history as far as available.

Results: Dox concentration measured from 2 cases was well within a concentration range considered therapeutic, whereas undertreatment may have occurred in another 2 cases. There were 2 cases of fatal Dox ingestion, as well as a case of high dosage and advanced putrefaction, respectively. Expectedly, concentrations present in heart blood were higher than those in femoral blood. Levels of both Dox and NDox were highest in lungs and lowest in muscle tissue among tissue samples. The molar Dox/NDox ratio in brain was significantly different from 1.0 in a single case, only.

Conclusions: Determination of the N-desmethyl metabolite along with its parent is recommended and analysis should include more than a single specimen. There was a trend towards a higher concentration sum in the brain with increasing combined levels in blood. The liver concentration sum may valuably be considered if a fatal ingestion can not be clearly separated from a person's medication usage. High concentrations present in lung tissue, and combined concentrations of Dox and NDox may also be helpful in making a cause of death determination. Overall, the sum of the absolute figures allows a more accurate interpretation in Dox related deaths as compared to the molar concentration ratio which may be helpful in acute ingestion.

Keywords: doxepin, nordoxepin, concentration ratio, *post mortem*

P013. *Post mortem* histological and toxicokinetic analysis evidence the failure of decontamination measures, anti-inflammatory and immunosuppressive therapy in paraquat human intoxications

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Introduction: Fatalities resulting from paraquat (PQ) self-poisonings represent a major burden of this herbicide. Specific therapeutic approaches have been followed to interrupt its toxic pathway, namely decontamination measures to prevent PQ absorption and to increase its excretion from organism, as well as the administration of anti-inflammatory and immunosuppressive drugs. Until now, none of the *post mortem* studies resulting from human PQ poisonings have assessed the relationship of these therapeutic measures with PQ toxicokinetics and related histophatological lesions, these being the aims of the present study.

Methods: During 2008, we collected human fluids and tissues from five forensic autopsies following fatal PQ poisonings. PQ levels were measured by gas chromatography-mass spectrometry. Structural inflammatory lesions were evaluated by histological and immunohistochemistry analyses. Photodocumentation was also obtained.

Results: The samples of whole blood, urine, gastric mucosa, duodenal wall, liver, lung, kidney, heart and diaphragm, showed quantifiable levels of PQ 9 hours to 6 days post-intoxication. Structural analysis showed diffused necrotic areas, intense macrophage activation and leukocyte infiltration in all analyzed tissues. By immunohistochemistry it was possible to observe a strong nuclear factor (NF)-κB activation and excessive collagen deposition.

Conclusion: Considering that 9 hours to 6 days after intoxication, PQ is still present in all tissues and the inflammatory reaction that ultimately leads to fibrosis was manifested, we conclude that the therapeutic protocol usually performed needs to be refined, in order to increase the efficacy of PQ elimination from the body as well as of the anti-inflammatory measures.

Keywords: paraquat, humans, *post mortem*, toxicokinetics, inflammation

P014. Free and total morphine blood concentrations ratio and drug addiction

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Introduction: Opioid overdose remains one of the major causes of death among heroin users. The factors contributing to opioid-related deaths, however, remain poorly understood. Tolerance, drug addiction and use of other drugs probably could play an important role. One of the most important factors influencing heroin death is the degree of drug addiction developed by the heroin users. However, the degree of drug addiction of heroin overdose victims is generally unknown. While is well recognized that tolerance and drug addiction represent risk factors for heroin death, limited data are available about the mechanism of their interaction. It has been shown that chronic exposure to heroin causes a relative increase of the active morphine metabolite morphine 6-glucuronide. Aim of the study was to analyse the relationship between drug addiction and heroin death using analytical data obtained from cases of heroin death. Hair analysis could be useful to discriminate between an addicted and no addicted subjects, but it can not help to understand the kind of pharmacological effects. The ratio of free and total morphine can assist for this purpose.

Aim: Aim of the present work was to study if there is a relationship between the free and total morphine concentrations ratio and drug addiction.

Methods: The cases were "certainly fatal" heroin overdose examined at the Toxicological Laboratory of Macerata from 2005 to 2008. The victims of intoxication for morphine alone (53) were divided into the addicted (AD; n=36) and non addicted group (NAD, n=17) on the evaluation of the presence or absence of morphine in hair, with assessment obtained by police or public prosecutors records. The samples were prepared for Gas chromatography/mass spectrometry analysis by solid phase extractions. In all cases were analyzed free (FM) and total blood (TM), urine (UM) and bile (BM) morphine concentrations.

Results: The results show that the FM/TM ratio of the AD group was significantly lower than that of the NAD group (0.59 mg/l versus 0.79 mg/l, $p<0.001$). On the contrary, although both TM and FM values of the NA

group were higher than those of NAD group (0, 633 mg/l versus 0.47 mg/l and 0.29 mg/l respectively) these differences did not reach statistical differences. The results show statistical differences only for what concern bile concentration (AD=27.53 mg/l versus NAD=16.04 mg/l, $p<0.05$).

Conclusion: Our results could be explained on the basic of a pharmacokinetics or pharmacodynamic interaction between drug addiction and heroin death. The decreased FM/TM of the AD group and the absence of differences in urinary excretion respect the NAD group could suggest that a pharmacokinetics interaction could be prevalent. Chronic exposure to heroin could cause a relative increase in metabolism with a consequent reduction of free morphine concentrations.

Keywords: heroin death, drug addiction, free and total blood morphine concentrations ratio

P015. Patterns of volatiles detected during forensic ethanol analysis in autopsy blood samples from violent deaths

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Introduction: The detection of volatile compounds in *post mortem* blood samples could be the result of either the *ante mortem* consumption of alcoholic beverages (ethanol, 1-propanol, 2-propanol, and acetone) or physiological metabolic processes (acetaldehyde, acetone, 2-propanol) or of *post mortem* production due to the microbial activity (ethanol, acetaldehyde, 1-propanol, 2-propanol, acetone). However, data concerning the concentrations of these volatiles in autopsy blood samples are still not available.

Aim: The aim of this study was the quantitation of ethanol and the volatiles, acetaldehyde, 1-propanol, 2-propanol and acetone, in *post mortem* blood samples sampled during autopsy from cases classified as violent deaths. The volatile levels were classified according to (i) the presence or not of putrefaction at autopsy and (ii) the blood ethanol concentration (BAC).

Materials and Methods: *Post mortem* blood samples were collected from the femoral vein during autopsy at the Laboratory of Forensic Medicine and Toxicology, Medical School, University of Ioannina. The samples were collected in sterilized blood collection tubes containing anticoagulant and preservative and analyzed 2-8 h post autopsy by HS-GC-FID. HS-GC was performed using a Shimadzu GC 17A GC equipped with a SUPELCOWAXTM-10 fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm) and with a FID. All samples were measured in duplicates.

Results and Discussion: *Post mortem* blood samples from 239 routinely autopsied cadavers which were classified as violent deaths were quantitatively analyzed by HS-GC for ethanol, acetaldehyde, acetone, 1- and 2-propanol. (i) Cases were classified according to the putrefaction state at autopsy to cases with (14 cases, 5.9%) and without obvious (223 cases, 93.3%) putrefaction. In cases with putrefaction where the microbial activity was more probable to produce ethanol, BAC ranged from 0-208 mg/dL. Acetaldehyde, acetone and 2-propanol concentrations showed wide ranges irrespectively of the presence or not of putrefaction. The levels of 1-propanol were increased [(1.87±4.09) mg/dL] in cases with presence of putrefaction in comparison with [(0.13±0.65) mg/dL] the cases without putrefaction. (ii) The classification according to the BAC was made in cases with: BAC<10 mg/dL (171/239, 71.5%), BAC from 10-100 mg/dL (26/239, 10.9%) and BAC>100 mg/dL (40/239, 16.7%). The lower volatile levels were recorded for samples having BACs < 10 mg/dL. Acetaldehyde, acetone and 2-propanol showed wide concentration range irrespectively of the BACs. Our results are in concordance with previous studies which have reported wide dispersion of blood acetone and 2-propanol concentrations for drinking drivers, diabetics and fasting individuals. The concentration of 1-propanol was higher for the samples with BAC ranging from 10-100 mg/dL where the majority of samples with putrefaction were also including.

Conclusions: *Post mortem* blood concentrations of acetaldehyde, acetone and 2-propanol show wide dispersion in cases from violent deaths irrespectively of the presence of putrefaction or the BACs. *Post mortem* blood 1-propanol concentrations were higher in cases with putrefaction compared to those without putrefaction.

P016. Serum/whole blood concentration ratio for ethyl glucuronide and ethyl sulphate

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Background: Serum/blood (S/B) concentration ratios for ethyl glucuronide (EtG) and ethyl sulphate (EtS) are missing in the literature and the aim of this study was to determine these ratios in samples from patients at admission to an alcohol rehabilitation clinic.

Materials and methods: Two blood samples were collected simultaneously, and EtG and EtS were analyzed in whole blood and serum respectively, using a liquid chromatography-mass spectrometry (LC-MS) method. Separate calibration standards were prepared in both whole blood and serum for the calculation of whole blood and serum concentrations, respectively. The vacutainer tube used for whole blood was identical with those used for routine samples at the Norwegian Institute of Public Health and contained 143 I.U (0.286 mg) heparin and 20 mg fluoride. The vacutainer tube was filled with 5 mL of blood, resulting in a very minor dilution factor (1:260). For the serum samples, a vacutainer tube without additives was filled with 5 mL of blood.

Results: 13 pairs of serum and whole blood were analyzed. The median concentration of EtG (n=13) in blood was 2.69 mg/L (range 0.13-5.53), and in serum 4.59 mg/L (range 0.25-9.81). Regarding the S/B ratio, the median value for EtG was 1.69 and the range was 1.33-1.90. There was no correlation between the absolute levels of EtG in blood or serum and the S/B ratio. The median concentration of EtS (n=13) in blood was 1.13 mg/L (range 0.10-1.82), and in serum 1.56 mg/L (range 0.11-2.64). Regarding the S/B ratio, the median value for EtS was 1.30 and the range was 1.08-1.47. There was no correlation between the absolute levels of EtS in blood or serum and the S/B ratio. The S/B ratio was significantly lower for EtS than for EtG ($p<0.001$), using paired samples statistics (paired samples t-test).

Conclusion: The higher concentrations of EtG and EtS in serum than in whole blood has to be considered when whole blood results obtained from forensic toxicology are compared to serum or plasma results from clinical laboratories.

P017. Case study: purified tetrodotoxin intoxication

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Introduction: Tetrodotoxin (TTX), a potent neurotoxin in puffer fish, is a powerful sodium channel blocker and extends irregular repetition of muscle constriction and relaxation by depolarization of both exposed and adjacent neurons.

Case history: Two middle-aged men, Victim P and K, respectively, were found dead in a car on the side of the expressway in Korea. Considering the circumstances, the police tentatively concluded that they took a poison accidentally. However, external and internal examinations of the bodies were normal and drugs were detected less than therapeutic concentrations in our screening. The pathologist assumed intoxication to be a cause of death but was not able to conclude the cause or manner.

Methods: TTX was analyzed in *post mortem* samples and other evidences such as a syringe and an unidentified vial from the scene by a mouse toxicity

test, FT-IR, GC/MS and LC/MS/MS. The C9-base of TTX was prepared in the gastric contents and identified by GC/MS. TTX was quantified in blood using LC/MS/MS.

Results: The mouse toxicity test in gastric contents and other evidences showed that the toxicant could be TTX because several intoxication symptoms were similar to those of TTX. TTX was identified in the scene evidences using FT-IR. In Victim P the TTX concentration in gastric contents was extremely high and that in peripheral blood was 1.93 µg/mL. Therefore, it was presumed that TTX was orally administrated to Victim P, which killed him shortly. The concentration of TTX in Victim K's gastric contents was very low, 0.55 µg/mL, and that in peripheral blood was 1.23 µg/mL. Considering the possibility of *post mortem* redistribution it was presumed that he was killed instantly by non-oral administration.

Conclusions: The TTX blood concentrations in the present case were abnormally higher than other published cases related to puffer fish intake, which implied that the victims took not puffer fish but purified TTX. The case reported here may be the first one about purified TTX intoxication.

Keywords: tetrodotoxin, *post mortem* samples, LC/MS/MS

P018. Methadone related deaths in Norway

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Introduction: Methadone maintenance therapy (MMT) was instituted on a nationwide basis in Norway in 1998. Methadone has been the most commonly used drug in the treatment of heroin addiction since this time, although sales figures have fallen over recent years with the increasing popularity of buprenorphine and buprenorphine combination drugs. Drug-related deaths are, however, still a significant problem. According to data collected by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Norway has the 4th highest rate of drug-related deaths in Europe, only surpassed by Estonia, Luxembourg and Denmark, respectively. Reports have suggested that methadone may be involved in a significant proportion of these deaths.

Aim: We wished to study the development in methadone deaths over the period 2000 to 2006. In addition, we wished to examine toxicological and medical data for the deceased.

Methods: Forensic autopsies where methadone was detected at toxicological analysis were collected from a database at the Division of Forensic Toxicology and Drug Abuse (DFTDA), Norwegian Institute of Public health. Data regarding the deceased were collected, including age, sex and toxicological findings. The national cause of death registry was used to obtain data regarding the cause of death in each case, while MMT patient registers were used to find out whether the deceased had previously been in a MMT program.

Results: We found 304 deaths with methadone detection over the studied period. From 2000 to 2006, the number of cases per year increased by approximately 4 times. The majority were male (79%) and the mean age at death was 36. Over 80% of deaths were classified as overdoses/drug-related. Methadone concentrations varied from 0.06 mg/L to 6.2 mg/L, with a median concentration of 0.43 mg/L. Other causes of death included disease, violent death and suicide. 28% of the deceased were found in OMT treatment registers and 22% had been in treatment at the time of death. A lower proportion of those in treatment were classified as drug-related deaths, while a greater proportion of those in treatment were female, with a higher mean age and median concentration of methadone at analysis (1 mg/L). As far as the toxicological findings are concerned, methadone was the only drug detected in only 4% of cases. Common findings included benzodiazepines (primarily diazepam and flunitrazepam), cannabis, amphetamine/methamphetamine, morphine and alcohol.

Conclusions: The number of methadone-related deaths has increased over the studied time period, although data from 2006 and preliminary data from 2007/2008 suggest that this has not continued but reached something of a plateau. Methadone was, in the great majority of cases, detected in addition to other substances and benzodiazepines were a common finding.

The majority of the deceased were not in MMT. This highlights the problem of diversion and the need for tighter regulation of the drug.

Keywords: methadone, MMT, opioid, overdose, toxicity

P019. Fatal overdose of clozapine

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Introduction : Clozapine is a well-proven antipsychotic agent with a wide atypical receptor profile. It is effective against both positive and negative symptoms, with sympatholytic, anticholinergic and antiserotoninergic side effects. Major symptoms in severe overdoses are impaired vigilance, agitation, delirium, tachycardia, seizures, electrocardiogram changes, pulmonary complications and renal disturbance. Clozapine increases heart rate in the majority of patients and around 25% of individuals on therapeutic doses develop a mean increase of 10-15 bpm. Seemingly, the main causes are anticholinergic vagal inhibition and an increase in circulating catecholamines caused by a-1 adrenergic blockade.

Method: We present a case of accidental death in a 99-year-old woman, who mistakenly received a 50 mg dose of Leponex (clozapine).

Results: Thirty minutes after the administration, blood pressure was 118/61 mmHg, heart rate was 85 bpm. One hour later, blood pressure was 108/58, heart rate was 85 bpm. One hour later, blood pressure was 171/101, heart rate was 101 bpm. Because of deterioration in her spontaneous respiration, the patient was endotracheally intubated and artificial respiration was applied. Gastric lavage could not be performed. All attempts to reanimate the patient did not lead to the clinical improvement and she died due to cardiac arrest after three hours of intensive care. An autopsy was performed at the University Center of Legal Medicine in Geneva. External examination was unremarkable. Internal examination showed congestion of internal organs and pulmonary oedema. Neuropathological investigation was negative. Histological examination showed moderate generalized congestion and broncho-aspiration of foreign material. Toxicological tests included blood ethanol levels and screening for common drugs and illegal substances by gas chromatography and mass spectrometry. Toxicological analysis showed midazolam (blood concentration 40 mg/l), which was administered to the patient at the hospital, and clozapine (blood concentration 140 mg/l), whose level was consistent with the administration of a 50 mg dose of clozapine.

Conclusions: The cause of death was determined to be clozapine intoxication.

Keywords: adverse drug reaction, clozapine, tachycardia, overdose

P020. Abnormal concentrations of clotiapine (Entumine®) and other psychotic drugs in *post mortem* biological samples in a case of suicide

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Introduction: A 36-year-old woman was found dead in countryside from apparent drug toxicity such as it was suggested by the several empty blisters surrounding the body.

Aims: The aim of the study was to define the specific drug(s) involved in the accident and to assess the time of death through the determination of the parent compounds and their metabolite(s) in post mortem biological samples.
Methods: Preliminary drug screening of urine and humor vitreous samples was performed with Triage8® (Biosite Diagnostic, San Diego, CA, USA), while blood screening was made out using a gas chromatograph Agilent 6890 coupled with a Agilent 5973N mass selective detector (GC/MS Agilent Technologies, Palo Alto, CA, USA). The specific blood quantification of clotiapine, citalopram and 10,11-dihydroxycarbazepine, the oxcarbazepine's active metabolite, was carried out in GC/MS using ethaverine as internal standard (I.S.). Finally, the determination of ethanol in blood was performed by GC/FID.
Results: Clotiapine, citalopram and 10,11-dihydroxycarbazepine, one of the major oxcarbazepine's metabolites, were detected in blood samples, while the drug screening of urine and humor vitreous together with the blood measurement of ethanol failed to reveal any positive findings.
According to the half life of clotiapine and citalopram equal to 8 and 36 hours, respectively, they were detected unmodified. In the case of oxcarbazepine, being the half-life of oxcarbazepine within few hours, the metabolite 10,11-dihydroxycarbazepine was measured.
Clotiapine, 10,11-dihydroxycarbazepine and citalopram were detected exclusively in blood sample at 1.3 µg/ml, 112 µg/ml and 0.017 µg/ml levels, respectively.
In the case of clotiapine, the level was 4-5 times higher with respect to those described in an other suicide case (0.2 to 0.34 µg/m) [1]. Even in the case of 10,11-dihydroxycarbazepine, blood concentration measured was much higher with respect to the toxic concentrations described in literature (45 µg/ml) [2]. On the contrary, citalopram blood concentration was similar to the therapeutic dose reported in literature (0.02-0.2 µg/ml) [2].
No signs of blunt trauma on the body were revealed.
Conclusions: Analyses demonstrated that death was due to oral ingestion of fatal amount of clotiapine and oxcarbazepine, while Citalopram was founded at therapeutic level. To the best of our knowledge, a such blood concentration in a case of suicide was not already described in literature. The abnormal drugs blood concentration together with the fact that they were undetectable in urine and humor vitreous confirmed that death happened in a very short time.
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P021. Lethal methemoglobinemia and automobile exhaust inhalation (case report)

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Introduction and aim: Inhalation of automobile exhaust gas often leads to death by CO intoxication. In some cases the measured carbon monoxide hemoglobin saturation level (COHb) in blood is considerably below what is considered to be lethal. The death in such cases has been attributed to a combination of a high CO2 and a low O2 tension. We question whether another mechanism, methemoglobinemia, could possibly be of importance in such deaths. Exhaust fumes contain nitrogen oxide gases (NOx) that by inhalation and absorption can result in acquired and severe methemoglobinemia. Reports mentioning methemoglobinemia in *post mortem* cases are surprisingly scarce, and only one earlier report has related exhaust gas deaths to this hypoxic condition. Methemoglobin (MetHb) results when the normally ferrous iron in the heme molecules of hemoglobin is oxidized to the ferric state, with resulting inability to reversibly bind oxygen, and also an unfavourable left shift in the oxygen dissociation curve of the residual hemoglobin. High-degree methemoglobinemia causes serious tissue hypoxia, leading to unconsciousness, arrhythmia and death.
Methods: A case history will be presented, of a young man found dead in a car equipped with a catalytic converter, with a hose leading exhaust from the engine to the interior of the car.
Results: Analysis of *post mortem* femoral blood revealed a moderately elevated COHb of 18% (HS-GC/atomic absorption spectrophotometry), and a high MetHb of 56% (six-wavelength IL682 CO-Oximeter). No ethanol, narcotics or drugs were detected.
Conclusion: The existing literature in this field, including stability of MetHb in *post mortem* blood and methodology, will be discussed. We postulate that this death could possibly be attributed to a combination of methemoglobinemia and a moderately high COHb concentration.
Keywords: methemoglobin, MetHb, hemoglobin, carbon monoxide intoxication, COHb, automobile exhaust gas, *post mortem* changes

P022. Practical aspects of the quantification of target compounds in *post mortem* specimens

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Introduction: Due to the lack of certified reference materials, the quantification of target compounds in the field of *post mortem* toxicology is a major challenge. When an isotope-labeled analogue of the target compound is available, it can be used as an internal standard and reliable quantitative results will be obtained within a relatively short period of time. However, in aconsiderable number of cases such an analogue is lacking, and the labor-intensive method of standard addition becomes necessary. Although a theoretical description of this method can be found in different sources, the practical aspects of achieving reliable quantitative results in *post mortem* toxicology have not been discussed. Therefore, the assets, drawbacks and pitfalls of standard addition will be presented using practical examples.
Aims: The aim of this presentation is to discuss the effort needed for reliable quantitative determinations in *post mortem* specimens, in cases where neither directly comparable certified reference matrices nor isotope labeled analogues of the target compounds are available.
Methods: Different concentration levels of the target compound were added to the homogenized sample. The homogenate was mixed with a phosphate buffer (pH 7.4), subjected to ultrasonic treatment, automated solid-phase extraction, acidic/basic separation, and GC-MS analysis. A calibration curve was constructed and the initial concentration of the target compound was determined by extrapolation.
Results: To obtain accurate quantitative results with the method of standard addition the following preconditions are indispensable: a representative homogenate of the sample; the possibility for correct measurement of weight and volume; and linearity throughout the entire range of added concentration levels for the analytical method applied. Moreover, the reproducibility of the whole procedure (*e.g.* sample extraction, GC-MS analysis...) and establishing this method of quantification as a routine procedure in the laboratory will ensure reliable results. The question of how many levels are necessary to get a justifiable confidence interval of the obtained quantitative results will also be addressed.
Conclusion: The method of standard addition allows for the reliable quantitative determination of target compounds in the field of *post mortem* toxicology if certain preconditions are met. Although a considerable workload is required to perform this method, its application is necessary in order to achieve accurate results in cases where isotope analogues are lacking; the extra time and expense involved is justified due to, among other things, the serious legal consequences of such cases.

P023. Detection of amitriptyline in autopsy samples collected 27 years after death

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Introduction: A case report involving a 50-year-old man died in 1980 is presented: after death the body was not submitted to autopsy and it was entombed in a cement loculus. In 2007, the body was exhumed and autopsy was performed. No information regard to therapeutic drug use was available. Mummified liver and right kidney, blood clot found in the axillary artery, and one head hair strand were collected during autopsy and submitted to analysis.

Methods: Systematic toxicological analysis consisting in SPE purification (acidic and alkaline extraction) and GC-MS analysis was performed on blood clot and homogenated liver and kidney. Hair sample divided into three segments (0-3 cm; 3-6 cm and 6-10 cm) was submitted to alkaline hydrolisis (NaOH 2 M), SPE purification and GC-MS analysis.

Results: Amitriptyline and nortriptyline were detected in liver: the concentrations were respectively 5.8 µg/g and 8.85 µg/g. These drugs were identified also in hair strand. Amitriptyline and nortriptyline concentrations in hair samples were respectively: 0.54 ng/mg and 2.21 ng/mg (segment 0-3 cm); 1.39 ng/mg and 1.86 ng/mg (segment 3-6 cm); 2.12 ng/mg and 2.56 ng/mg (segment 6-10 cm).

Conclusions: Amitriptyline and nortriptyline were identified in liver and hair many years after burial, in despite of decomposition. The identification of these drugs in liver sample and the subsequent determination in hair, demonstrate – 27 years after death – the chronic use of tryciclic antidepressant from the man.

Keywords: amitriptyline, mummified samples

P024. The 'blue stomach' case: determination of Patent Blue V (E131) in gummi candies and gastric contents by UPLC-MS/MS

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Introduction : A 2,5-yr old female child, without medical history of illness, was discovered dead at early morning in her bed. Autopsy findings were completely negative, excepted for a marked blue-green color of gastric contents and wall. Routine toxicological analysis was negative, as well as specific analyses targeted onto toxic plants (especially *Datura spp.*, present as an ornamental shrub in the parents' garden), organic pesticides or mineral toxicants. Further questioning of the mother revealed that the day before she died, her child had eaten Haribo Smurfs™ gummi candies before going to sleep. In order to demonstrate that the gastric discoloration was an artefact due to these candies and not to the ingestion of a putative toxicant, a rapid method was developed by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) for the identification of Patent Blue V (E131), an organic food dye responsible for the typical color of Haribo Smurfs™.

Methods : Gastric fluid from autopsy (50 µL) was deproteinized by acetonitrile (ACN, 0.5 mL) after addition of 200 ng prazepam as internal standard (IS). After centrifugation (10 500 g, 5 min), 20 µL of the supernatant were diluted with 180 µL of 0.1% HCOOH then 10 µL of this mixture were injected onto the column. Separation was achieved on an Acquity UPLC™ (Waters) C18 1.7 µm column (100 × 2.1 mm, i.d.), using a gradient of ACN/0.1% HCOOH at a flow rate of 0.5 mL/min. Analysis was completed in 6.0 min. Detection was performed by a Quattro Premier™ XE (Waters Micromass) tandem mass spectrometer set in positive electrospray mode, using multiple reaction monitoring (MRM) mode with the following transitions : 561.1 > 163.9, 478.9 and 542.9 (E131) and 325.2 > 271 (SI).

Results: Under these UPLC-MS/MS conditions, the average retention times were 2.67 min and 3.75 min for E131 and the IS, respectively. The limits of detection (LOD) and quantification (LOQ) were 0.1 and 0.2 ng/mL, respectively. Linearity was established from LOQ to 2000 ng/mL, and recoveries were found > 93%. Intra- and inter-day precision were less than 17% and accuracy ranged from 94% to 108%. A qualitative analysis of Haribo Smurfs™ samples was performed, confirming the presence of E131. Analysis of the gastric fluid from the deceased child was then achieved, allowing to detect E131 at a concentration of 675 ng/mL.

Conclusion: The UPLC-MS/MS method described herewith was found sensitive, specific and rapid for the unequivocal determination of Patent Blue V in the gastric contents. To our knowledge this is the first application of liquid chromatography/mass spectrometry for the analysis of an organic food dye in *post mortem* samples. Further development of such analyses may be of value in forensic situations, *e.g.* to ascertain or disprove postmortally the intake of a definite food or beverage by an individual. In the present case our method contributed to refute the hypothesis of a toxic origin of death, raised in view of the gastric discoloration. Other *post mortem* investigations (including histology and genetics) remained negative and the child's death was finally ruled natural of unknown origin.

Keywords: gastric contents, Patent blue V (E131), UPLC-MS/MS

P025. *Post mortem* toxicology: the incidence of antidepressants from 1980 to 2008

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Introduction: Antidepressant drugs (ADs) are currently the mainstay of treatment for almost all the mildest form of depression and their use has increased substantially in the last two decades. Besides the oldest tricyclic compounds (maprotiline, amytriptiline,...) newer antidepressants have been introduced (selective serotonin reuptake inhibitors SSRI), since '90.

Aim: This study was a retrospective epidemiological characterisation of forensic cases where ADs were detected in the period from 1980 to 2008.

Both lethal poisonings where ADs played a fundamental role, alone or in association with other drugs, and different causes of death where ADs were present, are considered.

Material and methods: The material is made up of 133 cases involving ADs that is the 4.6% of total cases examined at the Department of Legal Medicine, University of Pavia, during the years 1980-2008. The cases were subjected to a forensic autopsy and a toxicological screening for alcohol and drugs. The alcohol detection was carried out by head space gas chromatography (HS-GC) while the systematic toxicological analysis (STA) was performed by STAS OTTO method until 1995 and afterwards by the GC-MS method published by Polettini *et al.* [1]. Quantitative determination was performed by GC-NPD and GC-MS.

Results: In 28 cases the cause of death was poisoning from antidepressants: four tested positive only for ADs, while in 16 also benzodiazepines were detected in therapeutic and toxic levels. The last 8 tested positive for Ads besides alcohol and other drugs. Antipsychotic, benzodiazepines or other drugs were the main compounds responsible of the death in further 13 cases where ADs had a synergic role.

The cause of death of 92 cases, where ADs concentrations were within the therapeutic levels, occurred by: acute narcotism (27%), suicide (24%), cardiac failure (8%) other drugs poisoning (14%) and other causes (traffic accidents, homicides… 27%).

Conclusion: The study highlighted that the fatal poisoning by ADs occurred in 21% of total cases where ADs were identified, while in 9.7% ADs played a synergic role.
Suicide and cardiac failure represented the 32% of the causes of death in those cases where ADs were detected at therapeutic levels.
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Keywords: antidepressants, *post mortem* toxicology

P026. Dying to be thin? Two fatalities attributed to use of 2,4-dinitrophenol

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Introduction: 2,4-dinitrophenol (DNP) is used in the manufacture of dyes, wood preservatives, photographic developers, explosives and, historically, fungicides and herbicides. It has been abused as an anti-obesity agent as it causes weight loss via increased lipid metabolism, initiated by an uncoupling of mitochondrial oxidative phosphorylation. Signs and symptoms of exposure include; fatigue, thirst, sweating, nausea, vomiting, dizziness, vertigo and headache. The oral LDLO in humans is said to be 36 mg/kg. In a six month period (Aug 07-Feb 08) the Analytical Unit received two cases of suspected DNP overdose. The first was of a 99 kg, 26 year old female who was alleged to have been taking 500 mg of DNP everyday for the last 5 days, and 1 g the evening preceding her death. In excess of 1 kg of a yellow powdery substance was collected from the deceased's home. The second was a 108 kg, 46 year old male who had left a message of his intention to end his life. It is believed he had taken in excess of 3 g DNP. Both individuals had originally purchased the DNP from the Internet for the purpose of weight loss.
Aim: Our aim was to establish methodology for the detection and quantification of DNP in biological specimens, for the purpose of assisting the Coroner in determination of cause of death in these two cases.
Method: Duplicate calibrators/samples (100 µL) were prepared for analysis using acetonitrile (500 µL) protein precipitation, followed by dilution of the supernatant (100 µL) with 0.1% formic acid (1000 µL). The extract (25 µL) was analysed by liquid chromatography with tandem mass spectrometric (LC/MS/MS) detection. A Sciex API2000 triple quadrupole mass spectrometer equipped with a turbo-ion spray interface, held at 400 °C, was utilised. The method was run in negative ionisation mode and set to detect the precursor and product ion transitions m/z: 183.2/108.9, 122.9, 136.8 and 153.0. Separation was achieved using an Alltech Alltima C18 column (150 mm × 2.1 mm, 5 µm) maintained at 50 °C. The mobile phase used was 45% acetonitrile/de-ionised water/formic acid (95/5/0.1, v/v/v) and 55% de-ionised/formic acid (100/0.1, v/v), at a flow rate of 300 µL/min. DNP eluted after 4.3 minutes.
Results: Officers from the Counter Terrorism Explosives Division identified the yellow powder as DNP by portable infra-red spectroscopy. DNP was detected at concentrations of 99.3 mg/L in ante-mortem blood, and 6.7 mg/L and 1.8 mg/L in *post mortem* blood and vitreous humour in cases 1 and 2 respectively. Therapeutic concentrations of paracetamol (11 mg/L), dihydro-codeine (0.05 mg/L) and pseudoephedrine (0.2 mg/L) were also detected in the blood of case 2.
Conclusions: In both cases the cause of death was attributed to DNP intoxication. DNP concentrations in previously reported fatalities have ranged between 28 and 315 mg/L (n=4). Whilst the concentration detected in case 2 was below this, few fatalities associated with DNP use have been reported, so experience of fatal concentrations is limited. In the past DNP intoxication was largely the result of occupational exposure, but poisonings associated with weight loss appear to be on the increase. In a society obsessed with appearance the temptation to exceed any recommended dose, in an attempt to 'speed up' weight loss, is great. It is important that individuals are aware of the dangers associated with DNP use.
Keywords: 2,4-dinitrophenol (DNP)

P027. Demographics of suicide victims in Sweden in relation to their blood-alcohol concentration and the circumstances and manner of death

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Introduction: Toxicological analysis of blood and other body fluids from victims of suicide often reveal a high blood-alcohol concentration (BAC), which verifies consumption of alcohol before they took their own lives. The acute effects of alcohol include loss of inhibition, poor judgement, risk taking and impulsive behaviour, all of which might increase the propensity to commit suicide in certain predisposed individuals.
Aim: To evaluate the age and gender of suicide victims in Sweden (population 9.2 million) in relation to the BAC at autopsy and the manner and circumstances of the death.
Methods: The toxicology results from N = 11,441 suicides were reviewed using a forensic toxicology database (TOXBASE). The concentrations of alcohol and/other drugs in femoral venous blood were retrieved and compared with age and gender of the victims and the mode of death. The BAC was determined in femoral blood by headspace gas chromatography and the analytical cut-off concentration for reporting a positive result was 0.1 g/L.
Results: The suicides were classified as self poisonings (N = 2,462), hanging (N = 4,474), asphyxia by gas (N = 509), drowning (N = 803), shooting (N = 1307), fall from height (N = 632), self-inflicted cuts (N = 363) and other ways (N = 891). The victims were mainly men 71% compared with 29% women ($p<0.001$), although the mean age of victims was about the same 51 y ($p>0.05$). Among the men 36% had elevated BAC (>0.1 g/L) when they died compared with women, who had a positive BAC in 31% of cases. The table below gives information about age, gender, alcohol positive cases, and the BAC (mean, median and upper 97.5th percentile).

Method of suicide	Mean Age y (± SD)	M v F% Percent Alc +ive	BAC, g/L	median	mean	Upper 97.5th percentile BAC
Self poisoning	52 ± 17	47 v 53%	45%	1.1	1.3	3.4
Hanging	50 ± 19	79 v 21%	32%	1.2	1.3	2.9
Asphyxia by gas	47 ± 16	89 v 11%	51%	1.2	1.3	2.8
Drowning	60 ± 18	50 v 50%	32%	0.94	1.1	3.0
Shooting with firearm	55 ± 19	98 v 2%	38%	1.6	1.6	3.2
Fall from height	54 ± 22	63 v 37%	19%	1.4	1.4	2.9
Sharp-force injury	54 ± 18	82 v 18%	18%	0.64	1.0	3.3
Other ways	43 ± 17	69 v 31%	32%	1.5	1.5	3.6

Conclusion: Roughly 20-40% of people who commit suicide in Sweden had consumed alcohol before the event and some were heavily intoxicated, judging by the 97.5th percentile BAC (range 2.8-3.6 g/L). The proportion of men to women depended in part on the manner and circumstances of the death; with considerably more men killing themselves by use of a firearm (98%) or gas asphyxia (89%). Elevated BAC was most prevalent in drug-poisoning deaths (45%) and also in deaths by gas asphyxia (51%). Alcohol was least prevalent in falls from height (19%) and self-inflicted sharp force injury (18%). The concentrations of other drugs identified in the blood of suicide victims will be reported separately.
Keywords: alcohol, drugs, drunkenness, blood-alcohol, suicide

P028. Ten years of fatal intoxications in a Swedish forensic autopsy material

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Introduction: Self-poisoning is a common cause of suicides and suicide attempts worldwide. In Sweden, the National Board of Forensic Medicine investigates all unnatural deaths and specimens of blood and urine for toxicological analyses are sent to one accredited laboratory, with complete national coverage. All toxicological findings are entered into a database along with the age and gender of the deceased and the cause of death according to the responsible forensic pathologist's report.

Aim: To describe the demographics of victims of fatal intoxications and the current pattern of pharmacological substances, both licit and illicit, identified in femoral venous blood of the victims of poisoning.

Methods: Cases were selected from the forensic toxicology and forensic pathology databases based on the ICD-9 codes linked to the cause of death diagnoses made by the forensic pathologist. Over a 10-year period (1998-2007) all suicides, undetermined cases and accidents in which the cause of death was attributed to a fatal intoxication were evaluated.

Results: The number of fatal intoxications (N = 7464) varied between 700-800 per year. There were 2449 cases classified as suicides, 2475 as uncertain and 2540 as accidents. We found that the suicides and uncertain causes of death decreased whereas the number of cases regarded as accidental death increased almost 2-fold over the study period. Women were over-represented among suicide deaths, whereas in the groups classified as uncertain and accidents the men were 2-3 fold higher than women. The average age of the victims was 49 y with a gender difference of 6 y (men 46 y, women 52 y). Multiple-drug intoxications were the norm and in only 10% of cases was a single substance identified in the blood samples. The median number of substances per case was three. The drugs most frequently detected were morphine, dextropropoxyphene, codeine, diazepam and propiomazine. In deaths classified as suicides dextropropoxyphene and propiomazine predominated and the hypnotic zopiclone was also a common finding. In the drug poisoning deaths classified as accidents, morphine was highly prevalent along with codeine and diazepam. Among fatal intoxications in which the cause of death was undetermined, morphine, codeine, diazepam and dextropropoxyphene were the commonest toxicological findings. Moreover, ethanol and paracetamol were frequently identified in drug-related poisonings from victims of suicide, accidental overdose and undetermined manner of death.

Conclusion: Over a ten-year period the number of drug poisonings considered as suicides decreased and the number of accidental poisonings increased 2-fold. Poly-drug use was common in drug overdose deaths in Sweden, which tends to complicate interpretation, owing to adverse drug-drug and drug-alcohol interactions. The drugs most frequently encountered in femoral blood in poisoning deaths were morphine, dextropropoxyphene, codeine, diazepam and propiomazine. The concentrations of these substances were generally above normal therapeutic levels. Many of the morphine/codeine findings reflected heroin-related deaths. Another aspect to consider when drug-related poisoning deaths are interpreted is the person's genotype and whether the individual was an ultra-rapid or poor metabolizer of drugs via CYP450 enzymes.

Keywords: poisoning, intoxication, toxicology, suicide, drugs

P029. Death by transdermal patches: fentanyl acute poisoning

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Aim: A report of acute self-poisoning by a health worker through the use of 11 fentanyl transdermal patches (Durogesic®), in association with midazolam perfusion and the ingestion of zolpidem, phenobarbitone and alcohol.

Case: A 51-year-old woman, a pharmacist, was found dead. The forensic physician recorded the presence of 11 transdermal patches on her body and a perfusion device positioned at her right hand. Various drugs, whisky and a hand-written letter were found in the room. Veinous blood was collected and sent together with the perfusion device and others syringes to the toxicology laboratory.

Materials and methods: Blood and solutions from the syringes were extracted by organics solvants. Dry residues were resuspended in 100 µL of mobile phase (ammonium formate/acetonitrile) and analyzed on an UPLC/MS/MS System (Waters, France) equipped with an ACQUITY HSS T3 column and a TQD detector. Two detection modes were applied on each sample, as follows: a full scan detection and a tandem mode MS/MS in MRM that allows the sensitive detection of 160 molecules often involved in forensic toxicology.

Results and discussion: Blood concentrations were 0.65 g/L for alcohol, 38.8 ng/mL for fentanyl, 39.4 ng/mL for midazolam, 49.4 ng/mL for zolpidem and 11.5 mg/L for phenobarbitone. One syringe contained midazolam (5.2 mg/mL) and another contained thiopental (90 mg/mL).

The blood concentration of fentanyl was in the letal concentration range [1,2] and led to respiratory depression, hypotension and coma. The fentanyl induced-sedation was enhanced by the ingestion of alcohol. Moreover, the self-administration of phenobarbitone, midazolam and zolpidem also increased respiratory depression. A solution of thiopental was prepared by the woman, but was not taken.

Conclusion : As a pharmacist, this woman had the professional knowledge to allow her to commit suicide by acute poisoning, without suffering and in her sleep.

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P030. Crime or accident… fatal ingestion of buprenorphine by a 3 year-old child?

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Aim: The goal of this case report is to present a lethal paediatric intoxication by buprenorphine.

This presentation will impact the forensic community demonstrating the great difficulties to conclude about toxicological analysis if there is no data about pharmacokinetics and pharmacodynamics properties of the toxic compound, and the necessity of publication about lethal case.

Introduction: Buprenorphine is a semi-synthetic opioïd. It's a partial antagonist of morphine receptor. On sale under the name Subutex®, it is used in high dose in the treatment of the opioïd addiction. Numerous cases of fatal overdoses, resulting from inappropriate uses adults patients, are described in forensic literature. The cases of accidental poisoning in children are much more rarely described in literature. And to our knowledge, no fatal case has been reported until days. This case is the first forensic description of a fatal poisoning in children by buprenorphine.

Case report: December 30, 2007 in the morning, a girl, 3-years-old is led by his father to emergency department in a comatose state. Initial toxicology tests highlought presence of buprenorphine in her blood, urine and gastric contents and norbuprenorphine in blood and urine. Despite the emergency care, child has died less than 24 hours after his admission. Initial police investigations have determined that this child was the illegitimate daughter of his father who had custody over the past week. Most of the time, she was kept by the wife of his father, who said she did not know who she really was.

During the evening before his death, she vomited three times. Nevertheless, she had no other clinical effect. His father, who had found tablets of Xanax® in the bedroom of the child, asked whether she had taken drugs. She answered « no ». During the night his father, who was sleeping in the same bed, said she was unusually calm. In the morning when the father woke up the child was in coma and snored abnormally. Only then, he led her to the hospital. An autopsy carried out after a « transplantation of organs » authorized by the judge, was little contribution because it was very disturbed by the resuscitation cares and visceral explantation. In particular, it's revealed congestion and pulmonary edema in large quantity. The *post mortem* toxicology tests on bile and gastric contents have confirmed the presence of buprenorphine. The microscopic histological analysis has shown lesions of acute bronchial pneumonia associated with the presence of food materials in large quantities in the airways. It was therefore concluded that death had resulted from respiratory failure on massive inhalation of food. The cause of death has been determined but what about the manner? The results of toxicology tests were compared with data acquired from science to determine the time and quantity of product ingested. But the lack of pharmacologicals investigations failed to answer these questions. So it is impossible to know whether it is a banal domestic accident or a crime masked a perfect accident or if some one failed in the child monitoring.

Discussion and Conclusion: Fatal cases of buprenorphine pediatrics poisonings are exceptional. In this case, the specific context in which the death occurred was very suspect but forensic investigation will not be able to help justice. Over there this case show the limits of interpretation in spite of forensic investigations thorough and complete results with ante and post mortem interpretation. Rare case, particularly poisoning paediatrics cases by substances whose pharmacokinetics and pharmacodynamics properties are unknown for children would have to be published.

P031. A study of the relationship between metahemoglobin, carboxihemoglobin and hydrogen cyanide in fire victims: interpretation of results and comparison with other episodes

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Introduction: In a previous work, in order to evaluate the contribution of carbon monoxide (CO) and hydrogen cyanide (HCN) as toxic agent in fire causalities to the diagnosis of cause of death, a lethal index for CO and HCN was defined (LICO and LIHCN). Carboxihemoglobin (COHb), total hemoglobin (tHb), methahemoglobin (MetHb) and hydrogen cyanide (HCN) were quantified in victims' blood to elucidate the cause of the death.

Aim: The purpose of this work is to analyse the blood samples of forensic autopsy cases in a tragic polyurethane mattress fire which provoked the deaths of 32 convicts in a prison of Buenos Aires Province, Argentina in 2006.

Methods: The cadaveric blood samples were gently drawn by puncturing the femoral vein and analyzed by CO-oximeter system IL80 (for tHb, MetHb, CO), microdifusion (for HCN). Blood alcohol (ethanol) and drugs were examined by HS-GC-FID and GC-MS respectively.

Results: Saturation of COHb ranged from 10% to 43%, tHb from 2 to 19.7%, MetHb from 0.10 to 35.7% and HCN from 0.24 to 15 mg/L. These latter values were higher than the lethal levels reported in literature. Other toxic components routinely measured (ethanol, methanol, aldehydes and other volatile compounds) gave negative results in the 32 cases. Neither drugs of abuse nor psychotropic drugs were detected. The results indicated that death in the 32 fire victims was probably caused in part by HCN, generated during the extensive polyurethane decomposition provoked by a rapid increase in temperature. However in this massive intoxication we considered the influence of oxygen depletion and the formation of other volatile compounds such as NO or NO2 and pathological evidence that demonstrated that heat was not the cause of death in all victims. In addition, statistical analysis showed that%COHb and%MetHb in blood concentration were not independent variables with c2 = 11.12 (theoretic c2 = 4.09, degrees of freedom = 12, a=0.05). Aiming to obtain a relationship between these two variables, different ratios were analyzed. The most probable ratio between concentration of COHb and Met-Hb for our 32 cases was the exponential decay model: where a and b are constants. With a non-linear regression model, the value of a and b was estimated through the representation of [CO-Hb/MetHb] as a function of MetHb. A good fitting with the experimental data was achieved using the proposed equation. The values of a=107.28±4.79, b=0.935±0.06 and determination coefficient r2 =0.958. On the other hand, we found no correlation between HCN and MetHb in blood from the victims.

Conclusion: This is the first report that has calculated the relationship between COHb and MetHb in forensic blood samples. Also, we discuss other factors that could contribute to the formation of a lethal atmosphere caused by fire and comparison with other published fire episodes.

Keywords: carbon monoxide, methahemoglobin, hydrogen cyanide, fire deaths, massive exposition, CO-oximeter, microdifusion

P032. Manganese intoxication: cases of accidental ingestion in Catalonia during 2008

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Introduction: Several studies describe intoxication due to manganese (Mn) inhalation and long-term contact, which cause pulmonary injuries. Manganism (chronic Mn poisoning) is related to mental changes, non-specific anaemia and paresis. Occupational exposure to Mn has been linked to the majority of the reported cases, but not in those presented here, which are related with intake of high amounts of Mn. Under these circumstances, inefficient excretion can give rise to fatal consequences and eventually to death. In these cases intake was due to a misprint in the labelling of the compound, because it was labelled as magnesium sulphate (MgSO4; compound with laxative properties sold as a non-prescription drug) instead of manganese sulphate (MnSO4), leading to intoxications, one of which was lethal.

Aim: The main forensic objective for our laboratory was to determine the cause of death by analyzing blood, viscera and evidences found near the corpse. First, X-Ray diffraction (XRD) was used to confirm that the product labelled as MgSO4 was MnSO4. Then, the content of Mn in samples obtained from the corpse was quantified using atomic emission spectrophotometry by inductively coupled plasma (ICP-AES), and the measured values were examined in light of the known tolerances.

Methods: The white powder labelled as "Epsom Salts-Name of Pharmacy" with self-adhesive label was analyzed by XRD. Analysis was performed with a Bruker D-8 Advance diffractometer. Results were obtained using Cu radiation, with primary Göbbel mirror and energy dispersive SOL-X detector. Analytical conditions were step size of 0.05 (with 1 s timing per step), 40 kV and 40 mA.

The biological samples were digested by adding 6 mL of 65% HNO3 and 1 mL 30% H2O2, and incubated for 16 h at 90 ºC. After cooling, the tubes were centrifuged and the supernatant was quantified by using a Perkin Elmer (MA, USA, www.perkinelmer.com) Optima 3100XL axial viewing ICP-AES.

Results: The white powder analyzed by XDR was identified as hydrated manganese sulphate (MnSO4·xH2O) crystallized as Szmikite. Afterwards,

ICP-AES quantitative results obtained from the biological samples are shown in the following table:

SAMPLE	[Mn]	SAMPLE	[Mn]	SAMPLE	[Mn]
Blood	6650 µg/L	Pancreas	26200 µg/Kg	Lung	7470 µg/Kg
Fat	1740 µg/Kg	Bile	376000 µg/L	Liver	63400 µg/Kg
Muscle	6610 µg/Kg	Spleen	9600 µg/Kg	Heart	89400 µg/Kg
Kidney	25500 µg/Kg				

Conclusion: Results point out a massive ingestion of Mn. Normal daily ingestion is 1-10 mg of Mn and in this case the dose was 10.000 times higher. Consumption of MnSO4 at high doses cause irritation in mucous membranes of mouth, pharynx, oesophagus and gastrointestinal (GI) tract and also nausea, vomiting, stomach ache and GI alterations.

Normal concentration of Mn in blood varies between 4 and 14 µg/L and in serum between 0.15 and 2.65 µg/L. Results showed a concentration in blood of 6650 µg/L. This level can lead to death but not with the symptoms related to a chronic exposure and long-term illness. Accordingly, present case is related with massive intake leading to organic failure and death.

Keywords: manganese, ingest, DRX, ICP, intoxication

P033. Development of an analytical methodology for the determination of anticonvulsants in whole blood and urine using liquid-liquid extraction and gas chromatography/mass spectrometry

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Introduction: Anticonvulsants are a diverse group of central nervous system depressants, which act by different action mechanisms and can be used for criminal purposes such as homicides, suicides, robberies and sexual assaults. The main forensic impact anticonvulsants in Colombia are pentobarbital, phenobarbital, carbamazepine, valproic acid and phenytoin. Thus it was important to develop a methodology for the determination of these analytes in cases associated with these kinds of crimes and also in deaths associated with the lack of the drug in epilectic seizures.

Aims: To develop a simple and selective methodology for the determination of anticonvulsants in whole blood and urine using liquid-liquid extraction and gas chromatography/mass spectrometry (GC/MS).

Method: The spiked whole blood and urine were extracted using liquid-liquid extraction with 6 mL of a pH 3 phosphate buffer and 6 mL of dichloromethane followed by derivatization with 50 mL BSTF/TMCs and the final addition of 50 mL of hexane. GC/MS analysis was performed using an 7890A GC Agilent Technologies equipped with a HP-5MS 5% a of phenyl methyl siloxane (30 m × 250 mm × 250 mm) capillary column coupled to a 5975A Agilent mass detector.

Results: From all the conditions tested, the best results were obtained with the methodology described above. Using SCAN and SIM modes five anticonvulsants were well separated from each other while a blank extract from whole blood and urine gave no peaks that interfered with all anticonvulsants. The internal standard used was aprobarbital.

Conclusions: The method is simple, selective and yields good resolution. It requires just a 0.5 mL blood sample or a 1.0 mL urine sample and just a single extraction and analysis method which can greatly improve the response time. The importance of the methodology in forensic cases is obvious.

Keywords: anticonvulsants, liquid-liquid extraction, gas chromatography–mass spectrometry

New trends in analysis of drugs, metabolites and natural products

P034. Forensic toxicology screening for 725 compounds using a GC/MS/NPD system and automated spectral deconvolution software

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Introduction: With recent advances in GC/MS technology, there are several opportunities to substantially increase the number of targets screened for and simultaneously reduce the time required per sample. With the system described here, samples are screened for 725 compounds using automated spectral deconvolution and reporting software. Data review time is substantially reduced compared to conventional techniques. Post run bakeout of heavy matrix compounds is replaced with column backflushing, which is faster and reduces system maintenance. Run time is reduced by using a fast GC run (9.75 min injection to injection) and simultaneously collecting scan, SIM, and NPD data. The scan data is deconvoluted and used to identify any of the 725 target compounds. SIM data is used to look for select low level compounds not detectable in scan mode. The nitrogen response of the NPD is used to highlight non-target nitrogen compounds, identity confirmation, and can be used for quantitation if needed. Using extracts of whole blood samples, the system finds all the compounds detected by the conventional method in significantly less time.

Aim: The purpose of this work is to show the benefits achievable by combining several recent GC/MS techniques into the toxicology screening process. The goals are to: 1) decrease the GC/MS run time 2) decrease the data review time and 3) decrease the number of false positives and false negative identifications in the screening process.

Methods: Whole blood extracts prepared for GC/MS analysis were supplied by NMS Labs (Willow Grove, PA). The whole blood was prepared with a single step liquid/liquid extraction into a solvent, evaporated to dryness, and reconstituted in toluene at 1/10th volume. Samples were run on a quadrupole GC/MS system with electron impact ionization. The GC column effluent was divided between an NPD detector and the MS with a splitter device. The splitter also provides for solvent venting and post-run backflushing of the column. The system simultaneously collects the full scan, SIM, and NPD data. The scan data is deconvoluted and screens for the presence of 725 compounds. The SIM data is used to analyze for 25 compounds at lower levels, and the NPD is used for identity confirmation and highlighting non-target nitrogen containing compounds.

Results: Significant time savings were obtained by collecting all three data signals simultaneously in a shortened run. Backflushing further reduced analysis time by eliminating the need for long post run column baking. The most significant time savings came in data review, where the deconvolution software identified the compounds present in ~10 minutes. The conventional review process required at least ~1 hour and was more prone to false negatives, especially where matrix interferences were present. The SIM data was useful for low level compounds like fentanyl, and the NPD prevented missing non-target nitrogen containing compounds.

Conclusion: The combination of techniques used here demonstrates that GC/MS toxicology screening can be significantly improved in both productivity and accuracy. Of the techniques evaluated, automated spectral deconvolution software makes the largest improvement in the screening process.

Keywords: fast GC/MS/NPD, whole blood, deconvolution, backflushing

P035. Toxicology screening of whole blood extracts using GC-triple quadrupole-MS

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Introduction: Toxicology screening is challenging due to the need to look for large numbers of target compounds in samples that contain complex matrix interferences. GC/MS methods are widely used and accepted for this analysis. Full scan EI methods offer many advantages for screening such as unlimited numbers of targets, full spectrum identity confirmation, and library searching for identification of non-targets. For detection of drugs at lower levels, SIM mode is often used to improve the sensitivity. SIM is sometimes limited, however, by matrix interferences that can prevent detection of analytes at trace levels. For these analyses, GC/QQQ can be used to avoid the interferences. The extremely high selectivity and sensitivity with this approach allows detection of drugs down to sub-picogram levels with minimal matrix interferences. A significant advantage is that it can be used to routinely monitor for large numbers of compounds (up to a few hundred) in a single run. This work describes using GC/QQQ to detect trace levels of drugs in extracts of whole blood. The samples were previously analyzed using GC/MS with simultaneous full-scan, SIM and NPD detection with automated deconvolution software. GC/QQQ is shown to be a powerful complement to the GC/MS/NPD/DRS system for those cases where trace level detection and confirmation is required.

Aim: The purpose of our work was to show: 1) the advantages of GC/QQQ in analyses of trace toxicological compounds in whole blood extracts and 2) to compare it to a single quadrupole system.

Methods: Whole blood extracts prepared for GC/MS analysis were supplied by NMS Labs (Willow Grove, PA). The whole blood was prepared with a single step liquid/liquid extraction into a solvent, evaporated to dryness, and reconstituted in toluene at 1/10th volume. Samples were run on a GC/QQQ instrument with electron impact ionization in MRM mode. MRMs (four per compound) for 11 common drugs were monitored. Results from the GC/QQQ experiments were then compared with screening results for the same samples previously obtained on the GC/MS/NPD/DRS system.

Results: The GC/QQQ system exhibited low pg detection limits and high selectivity for all but one of the compounds tested. When compared to the GC/MS/NPD/DRS system, the GC/QQQ was able to detect analytes at substantially lower levels than scan mode and somewhat lower than SIM. The major advantage over SIM was in selectivity. MRMs could be chosen for each compound that eliminated interferences seen in SIM. The major advantage of the full scan information was the number of analytes that can be screened for and the ability to identify unknowns not included in the target list.

Conclusion: GC/QQQ can provide both high selectivity and high sensitivity for the analysis of drugs. Low-level detection and confirmation of large numbers of target drugs in blood extracts is possible in a single run. Combined with information from a single quadrupole screening instrument like the GC/MS/NPD/DRS system, a much more complete picture of each sample is now possible.

Keywords: GC/QQQ, GC/MS/MS, whole blood

P036. Evaluation of a UPLC®-TOF drug screening method to replace the Bio-Rad REMEDi HS drug profiling system

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Introduction: The decision of Bio-Rad to discontinue worldwide support of the REMEDi Drug Profiling System (DPS) has necessitated its replacement in 4 Hong Kong hospitals. In collaboration with laboratories in Denmark and the UK, we have developed a method for comprehensive urine drug screening using UPLC®-TOF mass spectrometry.

Aims: To assess the transferability of the developed method between the six collaborating laboratories and to evaluate performance against the REMEDi system using routine patient samples.

Methods: Drugs were separated on a UPLC system (Waters) using an ACQUITY HSS T3 column (2.1 × 100 mm, 1.8 mm) and analyzed using a LCT Premier XE (Waters). To evaluate the transferability of the method between the six systems, a standard solution containing 30 drugs (ranging in molecular weight and chromatographic properties) was used. ChromaLynx™ software was used to identify the drugs based on retention time (RT), mass accuracy, isotope distribution and the mass spectral pattern of both the protonated species and the fragments. A total of 996 clinical urine samples were analysed in parallel, by both the REMEDi and the UPLC-LCT method. To compare the number of drugs and metabolites being detected by both systems, a 'R' score of 1 was assigned to a sample when the REMEDi system detected a drug and/or its metabolite(s) in that patient sample; similarly a 'L' score of 1 was assigned when the UPLC-LCT method detected a drug and/or metabolite(s). To compare the overall efficiency of the two systems, an 'I' score (for improvement) of 1 was assigned when the UPLC-LCT method detected one drug and/or its metabolite(s) that were not detected by REMEDi; a 'D' score (for deficiency) of 1 was assigned when the UPLC-LCT method missed a drug and/or metabolites that was detected by the REMEDi DPS.

Results: The transferability of the UPLC-LCT method was assessed. RT were demonstrated to be highly reproducible between laboratories, with an average deviation of 0.12% from the mean RT for each of the 30 drugs. ChromaLynx™ calculated the spectral match factors (MF) for both protonated ions and their fragmentation in comparison to both the in-house library and the libraries prepared using the collaborators' instrumentation. The deviation of the MF against the average was acceptable at <9%, indicating the spectra were reproducible between instruments. The parallel run study, showed a total 'L' score of 2613 and total 'R' score of 1514, indicating that the UPLC-LCT method detected 1.7 times more drugs and/or metabolites. The total 'I' and 'D' scores were 1290 and 197 respectively, showing that the UPLC-LCT method had a significant improvement over the REMEDi system. In all hospitals, both 'L' and 'I' scores were significantly higher than the 'R' and 'D' scores.

Conclusion: We have developed a screening method based on UPLC-TOF technology. Identification is achieved by comparison of spectral data and RT to a prepared library. Accurate mass measurement and isotope distribution pattern allow the prediction of probable elemental composition. The analysis of ~1000 authentic specimens during a parallel run showed that the UPLC-TOF method was able to detect more drugs and metabolites in routine patient urine samples and demonstrated significant improvements over the REMEDi DPS system.

Keywords: Screening, TOF, REMEDi

P037. Identification of cannabinoid analogs as new type of designer drugs in herbal products

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Introduction: *Cannabis sativa* L. (cannabis, marijuana) is widely abused around the World because it contains psychoactive cannabinoids, such as Δ9-tetrahydrocannabinol. Recently, cannabis abuse has increased in Japan. Moreover, a number of herbal products are also distributed in the illegal drug market in Japan. It has been said that some herbal products caused cannabis-like effects after smoking. However, active components in the herbal products have not been identified.

Aim: The purpose of this study was first, to isolate the ingredients of herbal products and identify the compounds using LC-MS, GC-MS, high-resolution MS and nuclear magnetic resonance (NMR) analyses. The second purpose was to analyze these compounds in the herbal products using LC-MS and GC-MS and to research the distribution of such herbal products in Japan.

Methods: Forty-four herbal products being sold in Japan for their expected cannabis-like effects were purchased via the Internet. The products were extracted with methanol under ultrasonication. After centrifugation, the supernatant solution was passed through a centrifugal filter (0.45 μm), then analyzed by LC-ESI-MS and GC-EI-MS. The sample solution was separated using a UPLC HSS T3 column (2.1 i.d. × 100 mm, 1.8 μm) and using gradient elution with a mobile phase of 0.1% (v/v) formic acid in acetonitrile and water, delivered at 0.3 ml/min. For the isolation of compounds, the products were extracted with methanol under ultrasonication and evaporated to dryness. Repeated fractionation of the extracts by preparative silica gel TLC gave two compounds. The accurate MS spectra of the target compounds was measured using a direct analysis in real time (DART)-TOF-MS. Assignments of these compounds were made via 1H-NMR, 13C-NMR and two dimensional NMR spectroscopy.

Results: LC-MS and GC-MS analyses indicated that the products contained two major compounds. One was identified as a cannabinoid analog (1RS,3SR)-3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol **(1)**, which is a C8-homolog of a non-classical cannabinoid called CP-47,497. The other compound **(2)** was 1-pentyl-3-(1-naphthoyl)indole [or naphthalen-1-yl-(1-pentylindol-3-yl)methanone] being identical to JWH-018. These compounds were synthesized and reported as potent cannabinoid analogs possessing a pharmacological cannabimimetic activity. Additionally, oleamide (cis-9,10-octadecenoamide), which shows cannabinoid-like behavioral responses, was detected in some products. Compounds **1, 2** or oleamide were found in the 38 products.

Conclusion: New types of designer drugs, cannabimimetic compounds, were identified as adulterants in various herbal products [1, 2]. This situation alerts us to the fact that other cannabinoid analogs will be found as designer drugs or adulterants in non-controlled products as cannabis replacements in the near future. To avoid abuse caused by new drugs, such compounds should be continuously monitored through surveillance.

Reference: [1] Uchiyama, N. *et al.*, Chem Pharm Bull, 2009; 57(4): 439-441. [2] Uchiyama, N. *et al.*, Forensic Toxicol, 2009, online available, <http://www.springerlink.com/content/5422n31876766340>.

Keywords: cannabinoid analog, herbal product, designer drug

P038. Metabolic properties of N-OH-MDMA in rat and human

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Introduction: A N-hydroxyl analogue of 3,4-methylenedioxymethamphetamine (MDMA), N-hydroxy-MDMA (N-OH-MDMA) has been recently distributed as a new designer drug in Japan. Very little data is available as to the metabolic and pharmacological properties of N-OH-MDMA, although it was reported that its N-demethyl analogue, N-hydroxy-3,4-methylenedioxyamphetamine (N-OH-MDA) was mainly metabolized to MDA and no other major metabolites could be detected in rats.

Aim: The purpose of our work was first, to develop an analytical method for the determination of N-OH-MDMA and its N-dehydroxyl and N-demethyl metabolites (N-OH-MDA, MDMA and MDA) in rat plasma, urine and hair samples, and second, to investigate the metabolic properties of N-OH-MDMA in human liver.

Methods: After the i.p. administration of N-OH-MDMA to pigmented hairy rats (5 mg/kg/day, 10 days), N-OH-MDMA and the three metabolites in the rat plasma (0-360 min after the administration), in the urine (0-72 hr) and in the newly grown hair (4 weeks) were determined by ultra performance LC (UPLC)-MS/MS. The hair sample was extracted by 1-hr sonication and over-night soaking in 5 M HCl-MeOH (1:20). The plasma, urine and hair extract samples were purified using a solid-phase extraction procedure. For in vitro experiments, cryopreserved human hepatocytes were incubated with N-OH-MDMA. Pooled human liver fractions, recombinant CYP isoforms or P450 reductase were also incubated with N-OH-MDMA in 0.1M Kpi buffer (pH 7.4) with NADPH generating system. These reaction mixtures were stopped with acetonitrile/MeOH (1:1) and filtered prior to the injection for the UPLC-MS/MS analysis. The separation was achieved in 8 min on an ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 × 100 mm) in a 1% formic acid-acetonitrile by a linear gradient program. MRM was used in the positive mode of an ESI-MS/MS for the quantitative analysis.

Results: N-OH-MDMA very rapidly disappeared from the rat plasma (<15 min) and urine (<10 hr), and MDMA and MDA were detected as major metabolites. Most of the N-OH-MDMA was excreted into the rat urine as MDMA and MDA and the total excretion amounts of N-OH-MDMA, N-OH-MDA, MDMA and MDA (0-72 hr after the last administration) were <0.1, 0.8, 261 and 181 μg, respectively. In the rat hair samples collected 4 weeks after the first administration, N-OH-MDMA (0.03 ng/mg) and N-OH-MDA (0.13 mg/mg) were clearly detected as well as MDMA (149 ng/mg) and MDA (52 ng/mg). In human hepatocytes, N-OH-MDMA was mainly metabolized to MDMA and MDA approximately by 10% and 3%, respectively, after 2-hr incubation. N-dehydroxylations and N-demethylation of N-OH-MDMA were performed in human liver microsomal fractions but not in cytosolic fractions. Using recombinant CYP isoforms and P450 reductase, N-dehydroxylation of N-OH-MDMA was scarcely performed by these enzymes, while N-demethylation was mainly performed by CYP2D6, 2B6, 2E1, 3A4 and1A2.

Conclusion: It was difficult to detect a parent compound and other metabolites except MDMA and MDA in the plasma and the urine of the rats administered with N-OH MDMA. As to in vitro experiments; N-OH-MDMA was mainly metabolized to MDMA and MDA in human liver microsomes and some CYP isomers were involved in N-demethylation of N-OH-MDMA. The rapid and complete conversion of N-OH MDMA to MDMA and MDA may be related to the psychotropic effets of N-OH MDMA.

Keywords: N-OH-MDMA, N-dehydroxylation, N-demethylation, UPLC-MS/MS

P039. Simple and sensitive determination of arsenite and arsenate by electrospray ionization mass spectrometry

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Introduction: Toxicity of arsenic (As) increases in the approximate order: metallic As < organo As compounds < arsenate (AsV) < arsenite (AsIII) < arsine, although some methylated AsIII compounds are reported to be more toxic than AsIII. Many analytical techniques have been employed for various samples to separate and quantify these As species. Among them liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS) offers the advantages of high selectivity in determining its atomic mass m/z = 75 and high sensitivity with a limit of detection (LOD) of 0.14–0.33 mgL^{-1}, but the identification of chemical species is based entirely on agreement of the chromatographic retention time with that of the reference compound. In some chromatographic separations, highly toxic AsIII elutes with negligibly toxic arsenobetaine derived from seafoods or monomethylarsonic acid, a metabolite of AsIII excreted into urine.

Aim: Electrospray ionization (ESI)-MS provides a unique opportunity for the analysis of molecular forms of species. Inorganic AsIII and AsV, however, could not be quantified on ESI-MS sensitively, and only their qualitative data

were provided previously. We therefore intended to quantify inorganic AsIII and AsV sensitively by ESI-MS.

Methods: AsIII was reacted with a chelating agent, pyrrolidinedithiocarbamate (PDC, C4H8NCSS–) and tripyrrolidinedithiocarbamate-arsine, As(PDC)3, extracted with methyl isobutyl ketone (MIBK). A 1-mL aliquot of MIBK layer was directly injected into ESI-MS instrument without chromatographic separation. Methanol was used as a mobile phase at 0.2 $mLmin^{-1}$ and the capillary temperature was set at 250 °C. The electrospray voltage was set at 4.5 kV and multiplier voltage at 1.3 kV. Nitrogen was used as a sheath gas (469 kPa) and as an auxiliary gas (8 units). MS data were collected at m/z 340–390. The quantification in ESI-MS was performed by the integration of the peak area of As(PDC)2+, an ionized fragment of As(PDC)3, at m/z 367 ± 0.5 in selected ion monitoring. AsV was reduced to AsIII with thiosulfate, and then the total inorganic As was quantified as AsIII.

Results: Twenty transition metals such as Fe, Cu and Co, well known to form chelate complexes with PDC did not show their signals at the signal of As(PDC)2+. Also no signals were detected there from several organo As compounds such as mono-, di- and tri-methyl As, arsenobetaine and arsenocholine. Salts such as NaCl, NaNO3 and Na2SO4 up to 0.3 M did not lower the sensitivity of the detection. Present method was validated for the analysis of urine samples. The LOD of As was 0.22 mgL^{-1} using 10 mL of sample solution, and it is far below the permissible limit of As in drinking water, 10 mgL^{-1}, recommended by the WHO. Results were obtained in <10 min with a linear calibration range of 1–100 mgL^{-1}. AsIII and AsV in the reference materials SRM 2670a and 1643e were quantified.

Conclusion: A simple, rapid and sensitive method has been developed for the determination of AsIII and AsV using ESI-MS. The solvent extraction of As(PDC)3 has eliminated interfereing substances as well as matrix effects derived from urine that reduce sensitivity in any kinds of MS.

Keywords: arsenite, arsenate, ESI-MS, pyrrolidinedithiocarbamate

P040. Development of an IRMS technology for tracing gamma-hydroxybutyric acid (GHB)

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Introduction: Recreative use of γ-hydroxybutyric acid (GHB) and to a lesser extent GHB-facilitated sexual assaults ('date rape') are a relatively recent and stable phenomenon among European countries. However, recent surveys indicate that consumption of its chemical precursors, γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), is a growing trend among drug users due to several promoting factors. Indeed, synthesis of GHB with these starting materials is rather simple and, most remarkably, these precursors exhibit a rapid conversion into GHB upon direct oral consumption. Moreover, both products are readily available on the internet for a relatively cheap price. Also, GBL and 1,4-BD are not regulated under the legislation addressing psychotropic substances and their precursors. Altogether, there seems to be only scarce limiting factor to the increasing popularity of GHB, GBL and 1,4-BD consumption and manufacturing. Thus, there is a need to develop analytical means to help law authorities fight against use and trafficking of GHB and its precursors at all levels.

Aims: In forensic cases, presence of GHB or precursors is investigated in items seized at the premises, in the form of drug samples or spiked beverages, but also as biological samples (urine or blood) collected from drug users or sexual assault victims. Although these substances may be detected by conventional analytical methods, any linkage between trace and source is difficult to ascertain. However, as these substances can be synthesized through many different routes, using a diversity of chemicals and over all different continents, variations in their carbon isotopes content will certainly be observed. Thus, the aim of this research was to develop a source inference model to trace the origin of GHB or precursors using isotope ratio mass spectrometry (IRMS).

Methods: GHB, GBL and 1,4-BD drug samples were obtained from different police departments in Switzerland, from internet retailers of various countries and regular chemical suppliers. During a first phase, the δ13C-value of approximately 30 different GBL specimens has been determined by GC/C/IRMS after dilution in dichloromethane. Every sample has been spiked with e-caprolactone, as an internal standard, and tetradecanoic acid methyl esther of certified δ13C-value, as an internal calibrator for the CO2 pulses.

Results: δ13C-values ranging from -23.1‰ to -46‰ have been measured, thus significant differences in the carbon isotopic ratio have been observed between all GBL samples. Values obtained from multiple measurements over a period of several months have shown excellent reproducibility and robustness. Indeed, standard deviations for GBL, e-caprolactone and CO2 pulses are 0.18‰, 0.12‰ and 0.16‰ respectively. Therefore, accurate comparisons between samples could be undergone and chemometrics analysis applied to these data has allowed to predict the membership of all specimens to a relevant group or source.

Conclusions: A method for the measurement of the carbon isotopes content of GBL by GC/C/IRMS has been developed and applied to about 30 samples. A very wide range of values has been obtained, allowing to discriminate between samples of different origins by means of statistical analysis and link a specimen to a definite source. Such variations are likely to result from the large diversity of the synthesis paths and chemicals used in the process, as well as the geographical location of the manufacturers. These parameters are investigated at the present time, along with GHB and 1,4-BD δ13C measurements to refine this model and implement it for more complex matrices. Thus, profiling of these substances seems very promising from a forensic perspective.

Keywords: GHB, GC/C/IRMS, precursors, chemometrics

P041. Differentiation of compounds with identical molecular formulae by LC-TOFMS: venlafaxine and tramadol and their demethylated metabolites as an example

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Introduction: Compounds with identical molecular formulae cannot be differentiated from each other by accurate mass measurement with LC-TOFMS. Tramadol and the N- and O-demethylated metabolites of venlafaxine (C16H25NO2, 263.1885 Da), as well as N- and O-desmethyltramadol (C15H23NO2, 249.1729 Da), are examples of such compounds. Particularly, the identification of tramadol and O-desmethyl venlafaxine has been challenging because of chromatographic co-elution and the weak MS fragmentation of tramadol.

Aim: The aim of this study was to differentiate compounds with identical molecular formulae by LC-TOFMS using in source collision induced dissociation (ISCID) fragmentation combined with in silico fragment prediction.

Methods: ISCID conditions in LC-TOFMS were optimized for tramadol and venlafaxine and their demethylated metabolites by flow injection analysis of reference standard solutions (10 µg/ml). Fragments were predicted by ACD/MS Fragmenter 11.01 software. Experimental spectra of the reference standards were compared to predicted fragment patterns, in order to assign the compound specific fragments. *Post mortem* urine samples, previously found positive for tramadol and venlafaxine (ten samples each), were analyzed with the optimized ISCID methods to elucidate the method's feasibility in practice.

Results: Compounds with identical molecular formulae were readily separated by their retention time and characteristic MS fragments. Mean mass accuracy and isotopic pattern match (SigmaFit) values of the fragments were 8.1 ppm (0.87 mDa) and 0.0078, respectively (twenty samples). Mean mass accuracy for fragments with a molecular weight higher than 100 Da was 3.9 ppm (0.67 mDa). In ISCID analysis, four potential fragments in addition to the protonated molecule [M+H]+ were found for each compound. The characteristic fragments detected and also predicted by ACD/MS Fragmenter for O-desmethyl venlafaxine were m/z 133.0648 and 107.0491, corresponding to [M+H]+–C7H17NO and [M+H]+–C9H19NO, respectively. For N-desmethyl venlafaxine, the characteristic fragments were m/z 215.1430 and 147.0804, corresponding to [M+H]+–CH5N–H2O and [M+H]+–C6H15NO, respectively. Characteristic fragments for tramadol were not detected, but the three compounds with identical molecular formulae were differentiated from each other based on the fragments of N- and O-desmethyl venlafaxine. The characteristic fragments of O-desmethyl tramadol were m/z 187.1117 and 107.0491, corresponding to [M+H]+–C2H7N–H2O and [M+H]+–C8H17NO, respectively. For N-desmethyl tramadol, the characteristic fragments were m/z 201.1274 and 189.1274, corresponding to [M+H]+–C7H15NO and [M+H]+–C2H6N–H2O, respectively.

Conclusion: Tramadol and venlafaxine and their N- and O-demethylated metabolites with identical molecular formulae were identified by LC-TOFMS, using ISCID with accurate mass measurement of the fragments. The method proved to be feasible in practical analysis of urine samples. The fragmentation prediction software ACD/MS Fragmenter was found to be instrumental in the identification of fragments with accurate mass.

Keywords: tramadol, venlafaxine, demethylated metabolites, LC-TOFMS, ISCID, in silico, fragment prediction

P042. The rapid analysis of opiates from low volume whole blood samples by LC-MS/MS utilizing TurboFlow methods

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Introduction: The opiate morphine, and its derivatives, are medicines commonly used in therapy. However, the semi-synthetic opiate diacetylmorphine (heroin) is subject to wide abuse. After administration heroin is deacetylated very rapidly in plasma to its major active metabolite 6-monoacetylmorphine (6-MAM), producing euphoric effects. 6-MAM is further converted to morphine which is conjugated to produce morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) along with other minor ones. The forensic toxicologist is often asked to interpret results and possibly account for time of death in opiate (especially heroin) abuse cases. This task can be made easier if it is possible to identify and quantify the components such as 6-MAM, morphine, codeine, dihydrocodeine and the glucuronides in whole blood rather than in urine. The analysis of opiates in human whole blood by LC-MS/MS can be done routinely after rigorous sample cleanup.

Aim: To present a method that may quantitatively analyse opiate compounds present in whole blood utilizing a simple fast, low-volume extraction procedure followed by TurboFlow method-online extraction chromatography coupled with selected reaction monitoring tandem mass spectrometry.

Method: Horse blood was spiked with a mixture of opiates (codeine, morphine, 6-MAM, M3G, M6G) from 1 ng/mL to 500 ng/mL. The isotopically labelled internal standard (d6-codeine) was spiked into each sample at 50 ng/mL. 150 µL spiked whole blood was mixed with 200 µL acetonitrile, vortexed and then centrifuged. 10 µL of the supernatant was analysed. For the analysis, an online extraction system TranscendTM TLX-1 (Thermo Scientific) was coupled with a TSQ Quantum Ultra triple quadrupole MS (Thermo Scientific).

Results: All of the analytes were detected, with appropriate sensitivity, with the method developed. The calibration curves for morphine, codeine and M3G/M6G covered 10 – 500 ng/mL and for the 6-MAM metabolite the curve covered 1 – 50 ng/mL. Carryover was calculated at less than 1% for all analytes.

Conclusion: The use of a TurboFlow method (online extraction chromatography) with tandem MS/MS allowed the specific and sensitive analysis of various common opiates and their metabolites from a small volume of whole blood. The calibration curves for all analytes considered were linear over the concentration range. Since the method is ~ 4 minutes, 15 samples per hour may be completed and significant time is saved in the absence of SPE sample preparation.

The method enables the forensic toxicologist to produce a full picture of the opiates and metabolites in blood to assist with the determination of time of injection (presence of 6-MAM) and the detection of M3G and M6G to determine prior use or accumulation following heavy use.

Keywords: opiates, whole blood, LC-MS/MS, TurboFlow

P043. New approaches in ionisation and mass analysis to support drugs of abuse screening solutions in the clinical toxicology laboratory

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Introduction: Laser diode thermal desorption (LDTD) is an atmospheric pressure ionisation technique which utilises an infra-red laser and charge exchange ionisation using an APCI corona needle. This allows direct analysis from dried samples dispensed into specific 96-well plates (LazWell) often with little or no sample preparation. The LDTD source is easily affixed to a mass spectrometer (MS) and does not require a liquid chromatography (LC) pump or column. The total time of each sample analysis is ~ 7 seconds, and thus, a 96 well plate can be fully analysed in less than 12 min. This speed of analysis could prove extremely useful in laboratories where high-throughput, ease of use and result turn-around time for positive/negative screening workflows are required.

Aim: To describe and compare the analysis of ~ 30 drugs of abuse (DOA) in human urine with both liquid chromatography – electrospray ionisation and LDTD ionisation coupled with a triple quadrupole or high resolution/mass accurate mass spectrometer.

Methods: LC-MS analyses utilised an Accela U-HPLC pump coupled to both an Exactive (orbitrap) mass spectrometer (Thermo Scientific) and a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Scientific), fitted with a heated electrospray (HESI-II) ionisation probe and a Hypersil Gold PFP LC column. An LDTD (Phytronix Technologies) ionisation source was directly coupled to both an Exactive (orbitrap) mass spectrometer and a TSQ Quantum Access triple quadrupole mass spectrometer. All analyses were carried out in positive ion mode, and utilised selected reaction monitoring (SRM) on the triple quadrupole MS and full scan MS on the orbitrap MS. The data were further processed through ToxID™ software.

Results: Human urine was spiked with 30 DOA analytes over a concentration series 1 ng/mL – 1000 ng/mL. The samples analysed by LDTD were detected over the concentration series by both triple quadrupole and orbitrap mass spectrometers. Quantitative data analysis was performed for both HPLC-ESI and LDTD data sets and showed that both data were linear over the concentration range and reproducible.

Conclusions: LDTD is a fast ionisation technique which allows linear and precise quantitative data of drugs of abuse compounds to be analysed directly from dried human urine. No liquid chromatography is performed, thus, there is no sample carryover and, since sample analysis time is ~ 7 seconds, this is an efficient technique to quickly eliminate negatives and identify presumptive positives, for example in work-place drug testing. Additionally, the use of an Exactive (benchtop orbitrap) mass spectrometer provides full scan data that can be retrospectively analysed when necessary. More importantly the high scan rate capability of the Exactive instrument is shown to be compatible with

the narrow peak widths observed in ultra high performance chromatography (UHPLC) and LDTD analyses. In this approach, screening is not limited to the number of tandem MS spectra entries available in a library but simply the accurate mass ion list, which can easily be extended to include thousands of compounds.

P044. Gas chromatography-mass spectrometric analysis of chemical warfare agents using software NAGINATATM without authentic agents

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Introduction: In the crisis management against chemical warfare terrorism, on-site samples and casualty specimen are sent to specific laboratories, and examined for detecting and identifying chemical warfare agents (CWA's), degradation products, metabolites and related compounds by instrumental analysis after pretreatment. CWA's are extremely toxic and unstable, which causes the safety problem and handling difficulty. In addition, usage of CWA's is legally restricted by "the Treaty Prohibiting the Development, Production, Stockpiling and Use of Chemical Weapons and Mandating their Destruction". Thus, it is desirable to reduce the chance of standard substance handling as less as possible and to accelerate the analytical time. Using retention time and response factor stored in databases, a calibration locking database (CLD) method enables gas chromatography-mass spectrometric (GC-MS) identification and quantification without the routine usage of standard substance/solution. The method mechanism requires fixing analytical parameters to eliminate their adjustment or modification, and so the method provides us quick and simple operation. These features can remarkably improve the technical problems related to the CWAs analysis.

Aim: The aim is to apply the CLD method to CWA's analysis, and established the NAGINATATM database.

Methods: The analytical targets are 5 nerve gases, 5 blister agents, 2 vomit agents and 3 lacrymators. Agilent Technologies 6890 gas chromatograph-5973A mass spectrometer was used with DB-5MS column and temperature programs for oven and interface. Column pressure was adjuseted to be 21 min of retention time for chlorpyrifos methyl. DFTPP target tunning was adopted in elctron ionization and scan (m/z 35-550) data aquisition. Samples mixed with internal standards were subject to GC-MS, and slope values of the plots of the peak areas against the concentrations, retention time, relative strength of the ascertainer ions against the quantifier ion, and mass spectra were registered as the NAGINATATM database.

Results: The calibration curves of the CWA's were linear or quadratic with the correlation coefficients of more than 0.005 in the range of 1 to 200 mg/mL. The CWA's samples of 200 mg/mL were analyzed for 40 days. CWA's were well ascertained by the NAGINATATM GC-MS system, and quantified to be 150 to 300 mg/mL with the between-day standard errors of less than 1.4%. There was no deviation of the quantification values against CWA's spiked to dichloromethane extracts of pond water and soils collected in Kashiwa throughout 40 day measurement period.

Conclusion: GC-MS method with NAGINATATM software integrated of CWA's data enabled identification and quantification of CWA's without authentic samples.

Keywords: gas chromatography-mass spectrometry, database, method development, chemical warfare agents

P045. Bromo dragonfly: gives you wings?

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Introduction: Bromobenzodifuranyllisopropylamine also known as Bromo dragonfly (DOB-Dragonfly, ABDF, BrDF) is a synthetic psychedelic compound belonging to the phenethylamine family. It was synthesised in 1998 and began to emerge as a "street drug" in Sweden, Denmark, Germany, Australia and the USA by 2005. Its psychedelic effects are due to agonistic action on serotonin receptors, in particular the 5-HT2 sub-family. The list of effects associated with its use includes: hallucinations, muscle tension, memory loss, confusion, panic attacks and anxiety. Duration of action may vary from 12 up to 72 hours, although the onset of effects are delayed and may not occur for up to 6 hours. BrDF has been seen as a liquid and powder but also appears as a blotter and therefore can be mistaken for lysergic acid (LSD).

Aim: Serum, urine and a paper wrap were sent to the forensic toxicology service for analysis, following the admission of an 18 year old male to an emergency department (ED). He had consumed two powders: an unknown white powder and BrDF. The symptoms included severe agitation, tonic-clonic seizures and hallucinations, which developed 8 hours after ingestion.

Method: 100 µL of calibrator/sample, 25 µL of 0.1 mg/L Bromperidol (IS), 100 µL of 1 M sodium hydroxide and 1 mL of MTBE were combined and mixed for 15 minutes. Following centrifugation the organic phase was transferred to tubes containing 250 µL of 1% formic acid. The samples were then mixed for 10 minutes and centrifuged. The organic layer was removed and 25 µL of the formic acid layer was injected onto the LC/MS/MS system. The LC system consisted of a Perkin Elmer PE200 Series autosampler, pump and column oven. Chromatography was achieved using a Alltech Alltima C18 (150 × 2.1 mm, 5 µm) column maintained at 50 °C. The mobile phase consisting of methanol/de-ionised water/5M ammonium formate (80/20/0.1, v/v/v), was pumped at 250 µL/min. BrDF and the IS eluted after 2.31 and 2.24 minutes respectively. Detection was by tandem MS/MS (Sciex API2000) equipped with a turbo-ion spray interface held at 300 °C, in positive ionisation mode. The multiple reaction monitoring transitions for BrDF were m/z: 293.9/197.7 and m/z: 295.9/197.9 for the 79Br and 81Br isotopes respectively and m/z: 420.1/165.1 for the IS.

Results: Analysis of serum, urine and powder from the paper wrap using LC/MS/MS identified BrDF. The concentration of BrDF in serum was less than 5 ng/mL. Presence of the compound in the powder was also confirmed by gas chromatography mass spectrometry (GC/MS), ultraviolet visible spectrophotometry (UV/VIS) and thin layer chromatography (TLC). Routine screening of serum and urine samples also detected cannabinoids, ketamine and its metabolites. Lidocaine, lorazepam and midazolam were present as a result of treatment given in ED.

Conclusion: The presented case is an example of recreational poly-drug use. All described symptoms and delayed onset are likely to be associated with BrDF ingestion. The serum concentration of ketamine (20 ng/mL) was subtherapeutic. BrDF is a research compound with limited data, currently controlled only in Denmark and Sweden, which has entered the recreational drug market. The submission of solid dose material along with biological specimens facilitates the monitoring of new abused compounds. More comprehensive toxicological ED screening would allow the extent of novel designer drug use to be fully determined.

P046. Optimization of urinary morphine-3-glucoronide acid hydrolysis by means of design of experiments (Doe)

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Introduction: Method development is one of the most time-consuming tasks in forensic toxicology, as important human and material resources are often spent on this.

The design of experiments (DOE), in particular the factorial design, is a statistical tool that allows the planning of the whole procedure, aiming at investigating the effect of the controlled factors on the response. In addition, using a minimal number of experiments, the extent at which the several variables interact with each other can be also assessed and documented. To better illustrate this approach for method optimization, the urinary morphine-3-glucoronide acid hydrolysis will be discussed.

Aim: To demonstrate the applicability of the use of DOE to optimize method development in forensic toxicology, using the acidic hydrolysis of morphine-3-glucoronide in urine samples as example.

Methods: Morphine and its tri-deuterated analogue (used as internal standard) were extracted from urine samples by LLE (ToxiTubes® A), and the extracts were evaporated to dryness under a gentle nitrogen stream at 50 ºC. Derivatization was performed by sylilation at 80 ºC (25 minutes). Chromatographic analysis was done by gas chromatography-mass spectrometry in SIM mode.

Using peak area ratio as response, the variables that could influence the hydrolysis were investigated, including temperature (range 70-130 ºC), acid concentration (range 33-50%) and time (range 15-90 minutes). A factorial design 23 for the screening and a response surface methodology were applied.

Results: The factors which influenced response at a greater extent were temperature and its interaction both with time and acid concentration. Acid concentration by itself did not significantly influence the response. By multiple regression analysis of experimental data, a second order polynomial equation is obtained, and can be used to predict the best working conditions.

Conclusion: Experimental design takes simultaneously into account several variables, and seems to be the most convenient approach to obtain the optimal operational conditions with a reasonable number of experiments. By using this approach, in substitution of the one-factor-at-a-time approach, the best results can be obtained and resources optimized. This allows the saving of time and money, since other approaches are in general more time-consuming and laborious, and do not take into account the interactions between factors.

P047. Spice diamond: home fragrance or synthetic cannabinoid?

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Introduction: Spice is a herbal blend sold as an ambient fragrance in European Smart Shops and online. 'Spice drugs' first appeared on the European market in 2008, but are spreading fast thanks to their easy availability. Spice has already been banned in Austria, Germany, and France, whereas it is still sold freely in other countries such as Italy and Britain. It comes in three blends, Silver, Gold, and Diamond. The product labels list 15 natural components (including Indian Warrior, Blue Lotus and Maconha brava, dubbed false marijuana due to its effects); this may explain why Spice is still legally sold in several European countries. Recent studies of the blends have detected JWH018 - a synthetic cannabinoid - whose effect is more powerful and enduring than cannabinoids present in *cannabis sativa* - to which the psychoactive effects of Spice seem to be attributable. The failure by some countries to ban Spice may also depend on its composite nature. However, its effects on health are still unclear.

Aim: To identify the active principles of Spice Diamond purchased from a Smart Shop.

Methods: Active compounds were extracted with chloroform from 30 mg of Spice Diamond. Quality analyses included GC/MS and TLC purification. For GC/MS, 1 µl of the extract was injected into a Saturn 4D mass spectrometer using a Phenomenex Zebron ZB-Drug-1 column, whose mid-polarity stationary phase allows analyte separation from interfering compounds. The mass spectra were compared with Wiley 6 and NIST 98M libraries. 1 µl was injected into a Saturn 2000 system fitted with a Factor Four MS/MS column. Even NIST 2000 database was used to find matches. 100 µl of the extract was layed on a TLC plate (silica gel 60 F 254; n-hexane: diethylether 2:1 v/v) using 100 µl of a chloroform extract of marijuana and 100 µl of a chloroform extract of hashish as reference standards. The plate was read under UV light (254 nm) and tested with Marquis (H2SO4:CH2O 10:1), which stains cannabinols pink.

Results: TLC demonstrated a spot at Rf = 0.20, similar to the Rf of cannabinol (0.21) found in marijuana and hashish. GC/MS analysis demonstrated compounds such as α-tocopherol, β-tocopherol, vanillin, JWH018, and the strong presence of a 332 molecule whose molecular formula was C22 H36 O2. The structural formula matched none of the natural cannabinoids found in C. sativa. The molecule can be ascribed to the CP 47,497 family, whose members derive from a potent (greater receptor affinity than THC in vivo) synthetic cannabinoid, 9-nor-9β-hydroxyhexahydrocannabinol, developed in 1980 by Pfizer. Additional tests using the TLC purified extract allowed identification of both the non-derivatized and the silanized form.

Conclusion: Spice is probably not a harmless herbal blend, as claimed by its manufacturers. It contains one or more chemical substances of synthetic, cannabinoid-like nature, likely with a psychoactive action. The study lends support to the hypothesis that Spice Diamond actually contains a larger group of molecules, all of them cannabinoid analogues. It also emphasizes the dangerous nature of the new substance, whose long-term effects and risks have not yet been fully explored.

Keywords: Spice, cannabinoids, JWH018

P048. Determination of levetiracetam in whole blood by a column switching technique with monolithic column

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Introduction: The analytical methods employing an on-line solid phase extraction step rather than a liquid-liquid extraction or an off-line solid phase extraction have many advantages with respect to the latter methods. They do not require an evaporation step as the other methods, decreasing the risk of contamination of the sample or losses of the analyte during the sample treatment. Conditioning, washing and elution steps can be performed automatically. They are more reproducible, less time consuming and, generally, they have higher recovery. Monolithic columns are widely used for the determination of substances by directly injecting biological fluids that are generally plasma or serum. The main advantage they have over conventional columns is that they do not get easily clogged as they do not contain any type of filters. Moreover, monolithic columns are not as efficient as conventional columns and are available in significantly less variety. Monolithic columns have been used as extraction and preconcentration columns in addition to the typical off line use.

Aim: The purpose of this work is to develop a sensitive and reproducible HPLC method for determining levetiracetam in whole blood by solid phase extraction, on a monolithic column, after a simple deproteinization of the sample.

Methods: 0.5 ml of whole blood samples were added with 1.5 ml of methanol for a light deproteinization and then centrifuged at 10,000 g for 10 minutes. Ten microliters of supernatant were injected into an Onyx Monolithic C18 column for a preliminary cleanup. The mobile phase of the monolithic column consisted of pH 3.0 phosphate-acetonitrile buffer (96:4 v/v) at a flow rate of 1.0 ml/min. Two minutes after the injection the extraction column was connected to the analytical column for 3 minutes, and the analytes trapped in the extraction column eluted in the analytical column for the final analysis. The final analysis was performed with a C18 150 × 4.6 mm I.D. reversed-phase column at room temperature.

The mobile phase of the analytical column consisted of pH 3.0 phosphate-acetonitrile-methanol buffer (94:4:2, v/v) at a flow rate of 1.2 ml/min. The detection was carried out at 210 nm. Seven aliquots of whole blood were spiked with a stock solution of levetiracetam to obtain seven calibration samples containing 2.0, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0 mg/L of levetiracetam. The samples were analyzed and the peak areas of the levetiracetam fitted versus the sample concentration to obtain a calibration curve. The recovery was determined by comparing the peak areas of standard solutions extracted with the procedure just described, with the peak areas resulting from the same solutions injected directly into the analytical column. The complete cycle time was 20.0 min.

Results: The specificity of the method was tested by analyzing a blank sample without levetiracetam. No endogenous interferences were observed. The method was linear between the 2.0-100.0 mg/L range. The quantification limit was 2.0 mg/L. Recovery was 87%. Within-day coefficients of variation ranged from 3.5 to 6.1%; between-day coefficients of variation (at 25.0 mg/L) were 6.5%.

Conclusions: Monolithic columns have been demonstrated to be suitable as extraction columns for the analysis of levetiracetam in whole blood. Precision, accuracy and sensitivity of the column-switching method are similar to conventional assays. Most of the time-consuming manual sample preparation with solid-phase extraction or liquid–liquid extraction could be substituted with a solid-phase extraction on monolithic columns. The present method has been applied successfully for the determination of levetiracetam in forensic toxicology.

Keywords: levetiracetam, monolithic column, column switching

P049. Comparison of plasma and whole blood extraction using resin-based mixed-mode cation exchange SPE with LC-MS/MS analysis

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Introduction: The extraction of whole blood is becoming increasingly widespread in bio-analytical applications. This approach is gaining popularity due to the requirement to analyse the entire sample in order to avoid overlooking drugs or metabolites not present in the plasma or serum fraction alone. Resin-based mixed-mode cation exchange SPE is widely used for the extraction of basic drugs and provides clean extracts due to the dual retention mechanism afforded by the sorbents.

Aims: This poster will assess the performance of resin-based mixed-mode SPE, EVOLUTE CX for the extraction of whole blood compared to plasma.

Methods: Human plasma and whole blood (100 µL) was extracted using generic SPE methods throughout. Overall ion suppression experiments processed blank matrix through the SPE, reduced to dryness and reconstituted in mobile phase spiked with 1 µg/mL caffeine solution. Recovery experiments investigation matrix spiked with a suite of basic analytes and extracted through the SPE procedure. Extracts were evaporated to dryness and reconstituted in appropriate mobile phase for LC-MS/MS analysis. Chromatographic ion suppression experiments processed blank matrix through the SPE and reduced to dryness. Extracts were then reconstituted and spiked with various basic analytes.All samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode.

Results: Overall ion suppression comparing plasma and whole blood extracts was measured by flow-injection analysis (FIA). Extract cleanliness comparing calculated matrix factors shows more ion suppression when extracting whole blood relative to plasma. The recovery experiment showed consistently high recoveries from both whole blood and plasma. The majority of analytes exhibited recoveries greater than 80% with corresponding RSD's less than 10%. Chromatographic ion suppression indicated good consistency comparing extract cleanliness in terms of matrix factors between plasma and whole blood with all analytes showing calculated responses above 0.75. Full results will be shown in the final poster.

Conclusion: Mixed-mode SPE shows high, reproducible basic drug recoveries for plasma and whole blood, however, plasma samples result in slightly cleaner extracts.

P050. Extraction of benzodiazepines from various matrices using resin-based SPE and LC-MS/MS analysis

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Introduction: Benzodiazepines are a widely prescribed class of drugs known primarily for their sedative and hypnotic effects. Because of these effects they have found use as muscle relaxants, anticonvulsants, anxiolytics and for the treatment of various sleep disorders. This widespread use along with various forms of misuse has led to the necessity of rapid and reliable methods for their analysis and quantitation.

Aims: This poster will show the application of EVOLUTE ABN, a resin-based SPE sorbent, to the extraction of benzodiazepines from various human biological fluids.

Methods: Human plasma, urine and whole blood samples were spiked with various benzodiazepines and extracted using EVOLUTE ABN. 100 µL or 1 mL of matrix was extracted in the 25 mg/1 mL or 50 mg/3 mL formats, respectively. All extractions were performed using generic SPE protocols. The SPE extracts were evaporated to dryness and reconstituted in appropriate mobile phase for analysis. All samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode.

Results: The analyte suite consisted of 14 different benzodiazepines: alprazolam, alpha-hydroxyalprazolam, bromazepam, diazepam, nordiazepam, estazolam, flurazepam, flunitrazepam, lorazepam, midazolam, nitrazepam, oxazepam, temazepam and triazolam. Extractions in the 25 mg/1 mL (96-well format) using 100 µL of matrix showed analyte recoveries greater than 80% and corresponding RSD's below 10% for all three matrices tested. Scaling the procedure to enable the extraction of 1 mL of matrix in the 50 mg/3 ml format once again resulted in high reproducible recoveries. The majority of analytes again showed recoveries greater than 80%. A couple of analytes fell below 80% but overall the extraction of these benzodiazepines scaled well between formats.

Conclusion: This poster shows the application of EVOLUTE ABN to the extraction of a wide set of benzodiazepines from various matrices delivering recoveries in excess of 75% with corresponding RSD's below 10%. By scaling the extraction from the 25 mg/1 mL to the 50 mg/3 mL format it was possible to increase sample volumes to potentially enable lower limits of quantitation to be achieved.

P051. Extraction of cocaine and metabolites using resin-based mixed-mode cation exchange SPE with LC-MS/MS analysis

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Introduction: Cocaine is one of the most widely abused illicit drugs available and not confined to any particular socio-economic class. Available in various forms it is highly addictive, however, instantaneous euphoric effects has led

to huge popularity. This widespread misuse has led to the necessity of rapid and reliable methods for analysis and quantitation from various matrices. EVOLUTE CX is a resin-based mixed-mode strong cation exchange SPE sorbent designed for the extraction of basic drugs. The dual retention mechanism of hydrophobic interaction and strong cation exchange enables a rigorous interference wash regime resulting in cleaner final extracts.

Aims: This poster will show the application of EVOLUTE CX to the extraction of cocaine and its major metabolites from various human biological fluids.

Methods: Blank human plasma, urine and whole blood samples were spiked with cocaine and metabolites at various levels and extracted using EVOLUTE CX in the 50 mg/3 mL format. The generic method is based on a 50 mM ammonium acetate buffer at pH 5. Column conditioning takes place with 1 mL of methanol follow by 1 mL of buffer. 1 mL of matrix was pre-treated with buffer and extracted through the SPE procedure. The dual retention enables subsequent washes of buffer and methanol (both 1 mL). Analyte elution is afforded by 5% ammonium hydroxide in methanol. The SPE extracts were evaporated to dryness and reconstituted in appropriate mobile phase for analysis. All samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode.

Results: The analyte suite consisted of: cocaine, norcocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester and cocaethylene. Preliminary results showed excellent extraction efficiencies for all analytes. Recoveries greater than 80% with corresponding RSD's below 10% were observed at all levels tested. Full results will be shown in the final poster.

Conclusion: This poster shows the application of EVOLUTE CX to the extraction of cocaine and major metabolites from various matrices. High reproducible recoveries were obtained for all matrices tested.

P052. Rapid and sensitive determination of methylene-dioxylated amphetamines in forensic samples using derivatization reagent

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Introduction: Amphetamine derivatives have become trendy drugs of abuse because they are powerful stimulants of the central nervous system. There is dramatic increase in the abuse and recreational use of methylenedioxylated amphetamine derivatives in many countries, especially among young people. Development of rapid, selective and senzitive methods for identification and quantification of these compounds (MDA, MDMA, MDEA, MBDB) in different forensic samples would be of huge interest. Derivatization is particulary important for trace analyses of biological samples in order to increase sensitivity or selectivity.

Aim: The aim of our work is to develop the procedure for rapid and sensitive determination of these compounds in forensic samples with HPLC method, using 3,5 dinitrobenzoylchloride (DNB) such as derivatization reagent.

Method: The chromatographic system used consisted of ternary pump, UV-DAD (Model 9065, Varian). UV detector operated at 200 nm and the reliability of derivatization reagent was tested. Conditions of derivatization were tested, including the pH, the reaction time and the DNB concetration. A Lichrospher® 60 RP-select B 5 µm, 250 × 4,6 mm id column with appropriate guard column was used for separation of derivatives formed. The mobile phase was acetonitrile/acidified water with phosphoric acid (2.1). Stock solution of the compounds were prepared in metanol with concentration of 1.00 mg/ml and the mixture of them, by mixing the appropriate quantities of each stock solution was prepared and diluting with metanol to the working concentration of 0.025 mg/ml. The 3,5 DNB solutions were prepared daily by dissolving the pure compound in acetonitrile.

Results: After chromatographic separation, we obtained the following retention times for DNB-methylenedioxylated amphetamines: 3.8 min (MDA), 4.5 min (MDMA), 5.7 min (MDAE), 6.8 min (MBDB) and 12.8 min (3,5-DNB). Maximum analyte conversions were obtained for a reaction time of 5 min. The effect of the concentration of (3,5-DNB) on analyte conversion was examined within the range of 0.001-0.015 mol/L, using reaction time of 5 min. The results obtained demonstrated that the responses of analysed compounds reached maximum when we used (3,5-DNB) concentration of 0.005 mol/L.

Conclusions: The HPLC method of determination of methylenedioxylated amphetamine derivatives could be applied for analyses of different forensic samples,such urine, blood, hair, etc. The best results are obtained using reaction time of 5 min and concentration of derivatization agent of 0.005 mol/L.

Keywords: HPLC**,** methylenedioxylated amphetamines, 3,5-DNB

P053. Two cases of confirmed ingestion of the novel designer compounds: 4-methylmethcathinone (mephedrone) and 3-fluoromethcathinone

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Introduction: Cathinone is a pharmacologically active alkaloid (stimulant) extracted from theleaves of the Khat plant (*Catha edulis*). Cathinone (Cath) and methcathinone (MC) are controlled under the UK Misuse of Drugs Act. However, derivatives such as ethcathinone (EC), 4-methylmethcathinone (4-MMC or mephedrone) and 3-fluoromethcathinone (3-FMC), which are not controlled under current law, have been produced and marketed to satisfy the drug dance scene culture. The dose, effects and safety of these products have not been evaluated, and are only known from user's discussion forums on drug chat room websites.

Aim: Two cases were admitted to an inner-city hospital emergency department (ED) on separate days within a 2 week period. Case 1, a 30 year old male, presented with drowsiness (GCS 6/15), respiratory rate 20 per minute, heart rate 47 per minute and BP 140/80 mmHg. He was alert and orientated within 2 hours of presentation. He subsequently gave a history of ingestion of 1 g of mephedrone, GBL, "neo-doves" and "neo-blues". Case 2, a 22 year old male, presented after oral ingestion of 200 mg of mephedrone and subcutaneous injection of 3.8 g of mephedrone. On arrival in the ED he was agitated with 7 mm dilated pupils, heart rate 105 per minute and BP 177/111 mmHg; these features settled within 6 hours of presentation. Serum and urine from both cases were sent for analysis at St George's - University of London.

Methods: A screening method was developed for eight methcathinone related compounds (Cath, MC, EC, 4-MMC, 2-FMC, 3-FMC, 4-FMC and dimethylcathinone (DMC)). Derivatives of Cath and MC were synthesised in-house as secondary standards by Kingston University and purity established by NMR. 500 µL of urine or serum were extracted by liquid-liquid extraction and screened using gas chromatography with mass-spectrometric detection. Chromatographic separation of all derivatives was achieved over a 20 min run. The principle fragment ion for 4-MMC and 3-FMC was *m/z* 58. Confirmation of methcathinone derivatives was by acetylation with acetic anhydride. Liquid chromatography with tandem mass spectrometric detection was used to confirm and quantitate 4-MMC and 3-FMC in urine and serum. Quantitative and confirmatory multiple reaction monitoring (MRM) transitions for 4-MMC and 3-FMC were m/z: 178.2/160.1, 145.1, 119.2 and 182.2/164.0, 149.0, 123.0 respectively. An additional compound was seen associated with the standard and urine

sample containing 3-FMC. Preliminary investigation by NMR, MS and IR identified 3-fluoro*iso*methcathinone, a by-product of the synthesis of 3-FMC. Further investigation is required to establish its activity.

Results: Routine toxicological screening showed case 1 to be positive in urine for GBL, cyclizine (administered in ED) and 3-FMC. No drugs were found in the serum. Case 2 was positive for 4-MMC in urine and serum (0.15 mg/L). No therapeutic or toxic reference ranges are available for 4-MMC and 3-FMC. The cathinone derivatives were not available to purchase as certified reference standards, so quantitative results should only be used as a guide.

Conclusion: The clinical features seen in case 1 were consistent with GBL toxicity, and the 3-FMC was consistent with the history. Case 2 is the first known case of lone use of mephedrone. Clinicians and analytical toxicologists should be aware of the potential for use of these compounds in patients presenting with signs of sympathomimetic toxicity.

P054. Methcathinone derivatives: findings from test purchases of capsules and powders from the Internet

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Introduction: Cathinone is a pharmacologically active alkaloid (stimulant) extracted from the leaves of the Khat plant (*Catha edulis*). Khat leaves are chewed recreationally in Somali and Ethiopian communities to produce a mild high much like caffeine from tea and coffee. Human metabolism of cathinone produces cathine and norpseudoephedrine, structurally similar to Amphetamine and epinephrine. Cathinone (Cath) and methcathinone (MC) are controlled under the UK Misuse of Drugs Act. However, derivatives such as ethcathinone (EC), 4-methylmethcathinone (4-MMC) and 3-fluoromethcathinone (3-FMC), which are not controlled under current UK law, have been produced and marketed to satisfy the drug dance scene culture. The safety of these products has not been evaluated and they are often sold in products where the contents are not declared accurately.

Aim: Seven products, were purchased from the BioRepublik website, sold as 'legal alternatives to ecstasy'. They were described as 'Neorganics' and included two generations of products. The first being 'Neo-Dove 1'– "a well known, best seller, unique supplement designed especially to be the ultimate influence". 'Neo-Dove 2' is the next generation – "a superior supplement that will make you feel vital and happy". One powder, called 'Charge+' was sold as 'Novelty Bath Salts' and purchased from Everyonedoesit.com. Three products with similar names to those on the BioRepublick site were purchased from the Future Legals website. Each product was analysed to determine identifiable compounds.

Methods: A screening method was developed for eight methcathinone related compounds (Cath, MC, EC, 4-MMC, 2-FMC, 3-FMC, 4-FMC and dimethylcathinone (DMC)). Derivatives of Cath and MC were synthesised 'in-house' by Kingston University and purity established by NMR. The contents of capsules or powders were dissolved in methanol and analysed by gas chromatography with mass-spectrometric (GCMS) detection in scan mode. Chromatographic separation was achieved for all derivatives over a 20 min run. The principle fragment ions for MC, 4-MMC, 3-FMC and 4-FMC was *m/z* 58; for DMC and EC *m/z* 72 and 44; and for 2-FMC *m/z* 161, 132 and 91. Confirmation of methcathinone derivatives was performed by acetylation, using acetic anhydride, over a 20 min run. An additional compound was seen in all capsules and standard containing 3-FMC. Preliminary investigation by NMR, MS and IR identified it as 3-fluoro*iso*methcathinone, a by-product of the synthesis of 3-FMC. Further investigation is needed to determine if this compound is active.

Results: Four capsules contained both EC and 4-MMC, in addition to caffeine; six capsules and the powder contained 3-FMC, with three of these also containing caffeine. The products ordered from BioRepublik were packaged together labelled as 'Multivitamin' and, although the capsules were different colours, it was not possible to identify the individual products by name. The powder Charge+ was described 'Not for human comsumption'.

Conclusion: This market is adapting rapidly to changes in demand and legislation. Many similar products are sold openly in high street shops and legal high websites in packaging that does not reflect the contents, effects or uses. They are often sold under the disguise of a 'safe alternative to illicit drugs' despite not undergoing any quality control or evaluation of their safety in human subjects.

P055. Cannabinoid metabolite identification in meconium using a hybrid triple quadrupole/linear ion trap LC-MS/MS system

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Introduction: Cannabis (marijuana) is one of the most widely used illicit drugs in the world. In infants, meconium, the first feces, is often analyzed to detect prenatal cannabis exposure. Although 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) is the most common target analyte in meconium, other less prevalent metabolites, also found in adult biological matrices, have been found in significant quantities in this alternative matrix. Also, fetal metabolism may differ from adult pathways, producing unique biomarkers potentially useful for identifying cannabis-exposed children. Additionally, other plant cannabinoids, such as cannabinol or cannabidiol, may be important indicators of cannabis exposure in meconium.

Aims: Our research aim was to utilize the power of metabolite ID and liquid chromatography mass spectrometry (MS) to identify potential new biomarkers for prenatal cannabinoid exposure.

Methods: Cannabinoid-containing meconium specimens were homogenized in methanol and analytes isolated by reverse phase/anion exchange mixed mode solid phase extraction. Samples were analyzed by LC interfaced to a hybrid triple quadrupole-linear ion trap MS. Experiments to detect metabolites of THC, cannabinol, and cannibidiol were performed. Standards for known metabolites 8-hydroxy-THC, 11-hydroxy-THC, THCCOOH, and 8,11-dihydroxy-THC served as references for retention time and MSMS spectral interpretation. Selective triple quadrupole survey scans were employed to detect potential metabolites and trigger information dependent acquisition (IDA) of full scan ion trap MSMS spectra. Predictive MRM (pMRM) transitions and precursor ion scans were used as survey scans to detect metabolites at low concentrations. pMRM transitions are theoretical MRM transitions calculated from mass shifts of common biotransformations (oxidation, methylation, glucuronidation, etc.). The sensitive linear ion trap MSMS spectra guided structural elucidation and confirmation of the various metabolites.

Results: The LC-MS/MS IDA workflow identified several potential cannabinoid metabolites, including oxidations and glucuronidations. The observed glucuronidation of several metabolites reinforces the need to hydrolyze meconium to maximize cannabinoid detection until glucuronide standards are commercially available for direct analysis. The selectivity of precursor and MRM scans was important for detecting structurally-related metabolites in the complex meconium matrix.

Conclusion: Metabolite identification using a hybrid triple quadrupole-linear ion trap was demonstrated. This technique had the capability to detect and confirm metabolites, even in very complex matrices. Several metabolites were detected, including glucuronides, demonstrating that hydrolysis of meconium is necessary. The selectivity of the precursor and pMRM scans

was instrumental in detecting the metabolites, while the use of sensitive ion trap full scan MS/MS spectra was valuable for structural information and metabolite confirmation.

Keywords: LC-MS/MS, Cannabinoids, Metabolite Identification

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P056. Development of a new desorption/ionization interface at atmospheric pressure for application of modern mass spectrometry in forensic sciences

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Introduction: Mass spectrometry is one of the most relevant techniques in clinical and forensic toxicology. Its development and improvement are based on the invention and utilization of new ion sources, new ionization methods, new mass analyzers and new sample pre-treatment techniques. Currently, an item of interest is the "direct" sample analysis with null or minimum sample pre-treatment. Recently, a new desorption ionization method called DESI (desorption electrospray ionization) has been developed by the group of Cooks in 2004: by this new approach, the organic molecules present at sample surface can be ionized and analysed by mass spectrometry (MS), without requiring any sample pre-treatment.

Aim: We are developing an alternative desorption/ionization method witch uses a pneumatically assisted solvent spray without electric potential for *in vivo* sampling of living tissue surfaces and to identify drug and xenobiotic exposure.

Methods: During the investigation of the real mechanism involved in ions formation, we verified that the pneumatic contribution is preponderant to the obtained results. Hence, our new desorption/ionization interface uses only a spray of pure solvent with no high voltage on needle. A key aspect of this project, applied to several complex matrix, is the number of controllable operating parameters that can be investigated and optimized to obtain an efficient surface analysis. The most important variables are taken in consideration were the source geometry (the spray angle and the ion uptake angle, as well as the various distances in aligning the spray, sample and mass spectrometer) and the characteristic of sprayer (contents of the solvent spray and gas flow rate).

Results: Our new technical solution has been employed on an Agilent 1100 series MSD Trap system, originally equipped with an ESI standard source, and Thermo Finnigan LCQ-DUO ion trap mass spectrometer (LCQ-DUO MS), originally equipped with an APCI standard source. All measurements have been performed in positive and negative ionization conditions, varying capillary voltage, nebulizing gas pressure, drying gas flow and end plate temperature. Acquisition was in MS or multiple mass spectrometry mode (MSn). We have applied this techniques to compound identification, active principles and drug identification in direct tablet analysis, active principles and drug identification in vegetable species.

Conclusions: Future developments will be related to optimize the source geometry and choose the characteristics of sprayer to maximize ionization efficiency (*i.e.* acidity/alkalinity of the solvent, the pH of sprayed solution etc.) in relationship to surface sample characteristics. The direct analysis of analytes present on the original surfaces of interest can be potentially useful in the toxicological field for in vivo sampling of living tissue surfaces, to identify drug and xenobiotic exposure, besides the chemical imaging of spatial distribution of analytes onto sample surfaces.

Keywords: clinical and forensic toxicology, mass spectrometry, DESI, direct sample surfaces analysis, new desorption/ionization interface at atmospheric pressure.

Alcohol, drugs and driving

P057. The importance of ethyl glucuronide in hair as a biomarker for alcohol abuse

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Case report: A 54 years old man under treatment for alcohol related disorders caused a car accident in which two other men died. During autopsy, blood, urine, bile, brain fragments, gastric content and hair (length 3 cm) were collected. Specimens were submitted to toxicological screenings. Ethanol was detected in blood, urine and gastric content. Furthermore, in order to evaluate the frequency and the amount of ethanol assumption, ethyl glucuronide (EtG) was measured in blood, urine and hair.

Methods: Screening analyses for amphetamines, triciclic antidepressant, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates were performed by FPIA and EIA, while ethanol was detected by REA. Confirmation results on blood, urine and gastric content were obtained with GC-MS and LC-MS/MS, while ethanol concentration was determined by HS-GC-MS. EtG was detected in blood, urine and hair by LC-MS/MS in ESI (negative ionization) mode. D_5-EtG was chosen as the internal standard.

Results: Screening analyses turned out positive for antidepressants, benzodiazepines and ethanol. citalopram (antidepressant), delorazepam and lorazepam (benzodiazepines) and ethanol were quantified. Results, including concentrations of EtG, are summarized in table below.

Analytes	Methods	Blood (therapeutic range)	Urine	Gastric content	Hair (3 cm)
Citalopram	GC-MS	0.15 µg/mL (<0.30)	0.42 µg/mL	1.04 µg/g	-
Delorazepam	LC-MS/MS	<0.01 µg/mL (0.01-0.03)	<0.01 µg/mL	<0.01 µg/g	-
Lorazepam	LC-MS/MS	<0.01 µg/mL (0.02-0.25)	0.05 µg/mL	<0.01 µg/g	-
Ethanol	HS-GC-MS	2.94 g/L	3.24 g/L	2.86 mg/g	-
Ethyl glucuronide	LC-MS/MS	4.70 µg/mL	68 µg/mL	-	722 ng/g

Conclusions: The concentration levels found for citalopram, lorazepam and delorazepam were compatible with therapeutic assumption. The high ethanol concentrations found in different biological fluids, indicate that the driver was likely to be under the psychotropic effect of alcohol at the time of the accident. Furthermore, the high concentration of EtG in urine provide evidence of massive alcohol abuse in the hours (up to about 80 h) preceding the accident. Despite the driver was believed to be under treatment for alcohol addiction, the high level of EtG in hair demonstrated its chronic alcohol abuse in the previous months.

Keywords: Driving under influence, ethyl glucuronide (EtG), alcoholism markers, LC-MS/MS

P058. Determination of 19 drugs of abuse and metabolites in whole blood by high performance liquid chromatography-tandem mass spectrometry

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Introduction: The so-called zero-tolerance legislation regarding drugs of abuse in traffic was introduced in Denmark in July 2007. Consequently, it has become desirable to assemble some of the most frequent drugs of abuse in one multi target method to minimise costs and to optimize workflow.

Aim: A high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method has been developed and validated for the determination of nineteen drugs of abuse and metabolites and used in whole blood.

Methods: The following compounds were included: amphetamine, MDA, MDEA, MDMA, metamphetamine, cocaine, benzoylecgonine, morphine, 6-monoacetyl-morphine codeine, methadone, buprenorphine, norbuprenorphine, ketobemidone, tramadol, O-desmethyl-tramadol, zaleplone, zolpidem and zopiclone. 5 ml of phosphate buffer pH 4.1 were added to 500 µl whole blood. Solid phase extraction on Isolute Confirm HCX columns was performed with 4% ammonium hydroxide in acetonitrile as eluent. Deuterated analogues were used as internal standards for all analytes, except for ketobemidone and O-desmethyl-tramadol, for which benzoylecgonine-d_8 and tramadol-d_3, respectively, were used. The analytes were separated by a methanol gradient using HPLC (Agilent HPLC 1100) with a 3 × 100 mm Varian Pursuit 3 C_{18} column, 3 µm particle size and quantified by MS/MS (Waters Quattro Micro MS/MS) using multiple reaction monitoring (MRM) in positive mode. Two transitions were used for all analytes, except for tramadol and O-desmethyl-tramadol. The run time of the method was 35 min including equilibration time.

Results: For all analytes, responses were linear over the investigated range with $R^2 > 0.99$. One-point calibration was found to be adequate by validation, thereby saving analysis of multiple calibrators. The LOQs for the analytes ranged from 0.0005 to 0.01 mg/kg. Extraction recoveries of the analytes were from 34% to 97%, except for zaleplone (6%). Both inter-day and intra-day precision were less than 15% (20% at the LOQ) for all analytes, except buprenorphine, norburprenorphine and zaleplone (less than 18%). Accuracy (bias) was within ± 15% (±20% at the LOQ) for all analytes, except MDMA and O-desmethyl-tramadol (within ±19%). No ionsuppression or enhancement was seen in the region of the analytes as well as suppression from co-eluting analytes. Matrix effects were found to be less than 23% for all analytes, except zopiclone (64%). High and low quality control samples gave acceptable values, and the method has been tried in international proficiency test schemes with good results.

Conclusion: The present method provides a simple, specific and sensitive solution for the quantitation of some of the most frequent drugs of abuse and their metabolites in whole blood. The method has been successfully applied to 412 forensic cases from October 2008 to mid February 2009, of where 267 cases were related to zero-tolerance traffic legislation.

Keywords: Drugs of abuse, HPLC-MS/MS, whole blood

P059. Preparation and uncertainty of ethanol reference standards

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Introduction: Ethanol reference standards are widely used in the forensics industry for determination of blood alcohol content. Accuracy of the standards is critical to the quantitative determination of ethanol in samples. The manufacture, certification and uncertainty of Cerilliant Ethanol Standards are presented in this poster. ISO/IEC 17025 requires traceability and uncertainty to be reported for reference materials used in quantitative testing applications. ISO guide 34 provides a framework for certification of reference standards as certified reference materials (CRM) with comprehensive uncertainty. Uncertainty can only be properly calculated after undergoing a thorough evaluation of all possible contributing factors.

Aim: The purpose of this poster is to present the factors critical to accurate preparation, demonstration of traceability to an appropriate Stated Reference and establishment of the uncertainty associated with the stated value of an ethanol reference standard. Possible contributors to uncertainty during each aspect of the manufacturing and certification process will be explored. Approaches to reporting of uncertainty values will be compared.

Methods: Cerilliant certified ethanol standards are prepared gravimetrically and provided as single use standards in flame sealed ampoules. The standards are tested for homogeneity and certified for concentration and purity. Calibration and certification are traceable to SI through NIST. Every aspect of production and testing is controlled to reduce uncertainty. Uncertainty components include: purity of the raw material, preparation, analytical verification, homogeneity of the batch, and stability over time.

Results: Uncertainty of Cerilliant ethanol certified reference materials was determined by differential perturbation. The combined expanded uncertainty calculations included uncertainty of the gravimetrically prepared concentration, uncertainty of the analytically verified concentration, uncertainty of the replicate analyses, uncertainty associated with preparation of the calibration curve points, uncertainty from the analysis of the calibration curve, uncertainty of the between bottle homogeneity and uncertainty of long-term stability data gathered in real time by analysis of a lot at different test intervals. The certified value was assigned based on the average of the prepared and analytically verified concentrations. An expanded uncertainty was calculated to estimate a 95% confidence interval using a coverage factor of k=2.

Conclusions: The critical end use of ethanol reference standards requires that manufacturers properly identify sources of uncertainty, establish an accurate and reliable concentration value traceable to a recognized Stated Reference and report all associated measurement uncertainty in accordance with international guidelines. While ISO/IEC Guide 17025 and ISO Guide 34 require reporting of uncertainty and a framework for certification, vendor practices for controlling, calculating and reporting of uncertainty can vary widely. Therefore, a thorough understanding of vendor certification and uncertainty values is critical to ensure that a laboratory's test results are accurate and can withstand scrutiny in a court of law.

Keywords: certified reference materials, certified ethanol standards, uncertainty, ethanol, alcohol, ISO Guide 34, ISO/IEC 17025, traceable

P060. Driving under the influence of amphetamine-like drugs

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Introduction: Concerning relevant effects on driving performance, it cannot be realized offhand whether a person is affected or considerably impaired by stimulants. Usually expected are an overestimation of capacity, caused by exaggerated self-confidence, and a misconception of the current situation.

Aim and Methods: Positive amphetamine cases in connection with driving under the influence (DUI) were evaluated and correlations between substance concentrations in the plasma and documented psycho-physical achievement deficiency were generated.

Results: Amphetamine-like drugs (amphetamine, methamphetamine MDMA or MDE) were present in 1857 of 8709 cases of DUI (21.1 %) either alone or together with other licit or illicit drugs. In 338 cases, amphetamine was the only psychoactive substance in blood at mean median and highest concentrations of 0.18 mg/L and 1.05 mg/L, respectively. The users of amphetamine-type drugs were mainly men (92.8%) and tended to be younger than the whole collective (mean 26.3 *vs.* 28.4 y). The widespread opinion is, that, after the consumption of amphetamines, centrally stimulative effects with corresponding consequences on the safe driving are to be expected. In contrast to this a lot of cases were observed, where you would rather suggest an influence of centrally sedating substances considering the psycho-physical conditions. Relations between concentration and effect can not be established.

Conclusion: After the consumption of amphetamine-like drugs, persons usually become conspicuous in traffic during the acute phase of intoxication as well as in the following phase of exhaustion which is, therefore, highly important concerning the driving performance. The exhaustion reaction has to be assumed as drug-induced. For laymen this can not be associated mandatory with the proved drug admission (consumption of stimulants). However, competently advised, assuming a drug-induced exhaustion reaction, corresponding psycho-physical achievement deficiencies can prove a relative driving inability during the declining intoxication. For stimulants with varying symptoms, depending on the stage of intoxication, working out danger limit values will be especially difficult.

Keywords: amphetamine, driving under influence (DUI), exhaustion, driving ability

P061. Determination of cocaine and benzoylecgonine in cases of driving under the influence

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Introduction: Due to an *in vitro* decomposition of cocaine (Coc), usually an analytical detection in unstabilized blood taking systems is impossible. In cases of driving under the influence (DUI), one uses the evidence of the main metabolite benzoylecgonine (BE). In a few regions in Germany the local authorities use systems containing NaF for taking a blood sample. Caused by inhibition of esterases in the taken sample Coc is still detectable in blood samples. The aim of a study was an examination whether the use of stabilized blood taking systems will give a benefit for a later expert opinion in cases of DUI.

Aim and Methods: Cases of DUI with positive findings for BE as well as for BE together with Coc were evaluated. Substance concentrations in plasma were correlated with documented deficiencies in the psycho-physical performance.

Results: In 734 out of 1425 cases positive for cocaine-like substances besides BE a positive result was also given for Coc (51.5 %). The users of cocaine were mainly men (93.2 %) with a mean age of 29.0 y. If both substances were found (mean Coc concentration 83.6 ng/mL) the concentration of BE was significantly higher (mean 669 ng/mL) compared to cases with a single detection of BE (mean 209 ng/mL) (p=0.001). In 95 cases without any detection of further drugs cocaine users seem rather excited and stimulated towards intervening police officers, in particular when Coc is present in the blood test (17.8 %) taken afterwards. Also when evaluating medical investigation reports a rather stimulative effect (25 % *versus* 3.6 % sedated) is to be registered with presence of cocaine in the blood in a higher magnitude. Whereas with a sole determination of BE, a stimulated (19 %) as well as a sedated impression (14.9 %) is described. Definite concentration-effect relations can not be recognized. Indeed, more peculiarities are to be registered with the simultaneous detection of Coc than with a sole BE determination.

Conclusion: The determination of Coc and the differences in the BE concentration can be explained by the fact that the simultaneous detection of both substances is indicative for of a consumption shortly before the blood sampling. A sole detection of BE is more likely indicative for a consumption already some time ago. Therefore, in the first case one would rather suggest an acute intoxication phase. A determination of BE without Coc is more likely indicative for a transition to the drug-induced exhaustion phase which is also to be expected after the consumption of Coc. As with the amphetamines, there can be a psycho-physical loss of efficiency relevant for traffic during the acute intoxication phase as well as in the post acute phase. Drivers suffering from exhaustion also tended to drive less safely. The absence of Coc can be seen within the scope of a validity check as an indication of a possible exhaustion reaction. From the forensic-toxicological point of view a use of fluorid stabilized blood sampling systems is to be advised imperatively, because only then an analysis of the active Coc is possible. This makes it easier to investigate the state of intoxication or to appraise the temporal connection between Coc consumption and incident or blood sampling.

Keywords: cocaine, benzoylecgonine, driving under influence (DUI), exhaustion, driving ability, fluorid

P062. Prevalence of alcohol, licit and illicit drugs in traffic in South-East Hungary

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Introduction: One of the main aims of DRUID (driving under the influence of alcohol, drugs, and medicines) EU-6 project is to determine the prevalence of alcohol, certain licit and illicit drugs among car drivers in 13 European countries. As one of the participants, our department checks randomly stopped car drivers for alcohol and drug consumption in Csongrád county (Southern-East part of Hungary).

Aim: of this study is to determine the alcohol and drug prevalence in Csongrád county during a 2 years sampling period.

Methods: Oral fluid (OF) samples from randomly stopped drivers are collected by Statsure device in different seasons, urban and rural roads, different periods of the day, both on week days and week-ends. Breath alcohol is measured on the scene by the police (full cell method, Lion S-400), the OF samples are taken to the laboratory in cool bags and stored at –80 °C until analysis. 1 ml of OF samples are extracted with 5 ml butyl-acetate, the organic phase is evaporated, the residue is dissolved in acetonitrile and derivatized with MSTFA (morphine, 6-acetylmorphine, codeine, cocaine, methadone, THC, tramadol, ketamine) or with MTBSTFA (9 benzodiazepines, zopiclone, zolpidem), and analyzed with GC-MS (EI mode for illicit drugs, CI mode for benzodiazepines). Amphetamines are extracted from 0.2 ml OF with 0.5 ml toluene containing HFBA derivatizing agent, and measured in EI mode.

Results and discussion: The sample collection and analysis is still in progress and will be finished by the end of September 2009. On the basis of the samples have already been analyzed, 2.05% was positive for licit, 1.47% for illicit drugs, and only 0.59% for alcohol. It is known, that the rate of alcoholic influence decreased during the last decade in Hungary, but there was no former road side study to detect the prevalence of licit and illicit drugs for comparison. The results show a much higher frequency of illicit drugs than expected from the autopsy results of dead drivers.

Keywords: car drivers, alcohol, licit and illicit drugs, oral fluid, GC-MS

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P063. Development of a LC-MS-MS multi-target method for 29 drugs and metabolites in oral fluid

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Introduction: As a part of the ongoing European joint investigation DRUID (driving under the influence of drugs) oral fluid was collected to estimate the prevalence of drug use in drivers. In total, 6300 samples were collected with the Stature device, which consists of an absorption pad that, after being saturated with oral fluid, is placed in a tube with buffer solution. A 1:1 ratio was achieved as the 1 mL oral fluid collected was mixed with 1 mL of buffer.

Aim: The purpose of this work was to develop a fast and robust UPLC-MS/MS method for the analysis of the DRUID core substances and some additional drugs and metabolites commonly used in Sweden at their established cut-offs. An important requirement of the method was the use of several transitions to enable identification using ratios.

Methods: Twenty-nine drugs and metabolites were included. Samples were extracted on a Gilson ASPEC XL-4 robot in a speed of 80 samples within 6 hours. Aliquots of 0.4 ml saliva-buffer mixture were loaded and extracted on 130 mg bond elute certify columns. A Waters Quattro Premier XE tandem-quadruple MS combined with an Acquity UPLC was used for the analysis. High-resolution separation was performed by a linear gradient chromatography on a 50 × 2.1 mm i.d. HSS-T3 UPLC-column with 1.8 µm particles using mobile phases consisting of 5 mM ammonium acetate buffer, pH 5 (A) and methanol with 0.05% acetic acid (B). Two MRM-transitions were used for each compound and criteria for their relative area intensities were set for positive identification. The most intense transition was used for quantification. Nine deuterated internal standards with different chemical properties were selected for the quantification.
Results: After optimization of chromatographic selectivity and electrospray ionization matrix effects were minimized and sufficient sensitivity was achieved for all 29 analytes included. Extraction recovery was 80-90% for all analytes except for THC that showed a recovery close to 70%. Linear responses were found when calibrations was performed ranging in the range from cut-off to 250 ng/ml with cut-off levels at 1, 5, 10, 20, 25 or 50 ng/ml depending on requirements from DRUID. With few exceptions the accuracy was within 90-110% at cut-off. Morphine, buprenorphine and THC were the three analytes with most impact on the selection of method conditions. THC (cut-off 1 ng/ml) showed poor ionization in acetonitrile and therefore methanol was chosen as organic modifier even though better peak performance was seen for several analytes using acetonitrile. Morphine as the most poorly retained compound and the highly nonpolar THC as the latest eluting compound determined the gradient profile. Moreover, severe matrix effects from additives (Triton X-100) in the buffer of the sampling device were seen late in the chromatogram. Optimization of pH and gradient profile resulted in a chromatography where buprenorphine eluted before Triton X-100 and THC after. Transition ratios were stable over time and identification using a historic target ratio with acceptance ranges depending on relative abundance was successfully used. The analysis of the samples has just started and so far we have only found medications such as tramadol, zopiclone, nitrazepam and meprobamate.
Conclusion: Although several compromises had to be made during method development the DRUID cut-off levels were reached for all analytes and a fast and selective chromatography within 5 minutes was achieved. The automated SPE procedure was found to be he time-limiting step.
Keywords: UPLC-MS/MS, oral fluid, DRUID

P064. Effects of ethyl alcohol on eye movements. An automatic eye-tracking study

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Introduction: Saccades are fast eye movements that take place as the gaze is shifted from an object to another, to provide optimum image resolution in the central portion of the fovea. An antisaccade is a voluntary saccade of equal amplitude generated to inhibit a saccadic movement in the opposite direction. Successful performance on the antisaccade task requires the efficiency of a number of attention functions, particularly concentration and reflex inhibition, thereby providing an experimental measure of a subject's ability to inhibit potent responses. The effect of psychotropic substances on such movements are thus considered as a good measure of psychomotor ability, pharmacodynamic activity and drug interactions.
Aim: To investigate the effects of low alcohol concentrations on antisaccadic function using an automatic, non-invasive tool, and to test its applicability to the study of psychomotor performance alterations.
Methods: Twenty healthy volunteers, 8 male and 12 female, aged 18-65 years (mean 41.5) were recruited. A single dose of alcohol (0.5 g/Kg) or placebo was administered according to a double-blind, cross-over design. A 7-day wash-out period was applied between treatments.
A Tobii 1750 automatic eye-tracker equipped with a proprietary management software (ClearView, both from Tobii Technology AB) was used. The tests were held in a soundproof room with diffuse lighting. Subjects sat on a chair. Correct back inclination was continuously monitored by the eye-tracker. Light beams from LEDs embedded in the eye-tracker screen, trained on the centre of the pupils, enabled eye movements to be inferred from changes in the corneal reflex, whose direction is a function of pupil position (hence of gaze direction). An infrared light sensor at the base of the screen recorded the corneal reflexes; eye movements were recorded at a velocity of 50 Hz with an accuracy approaching an amplitude of 0.5° of the visual field. The software calculated precisely gaze direction and duration. Volunteers received the treatment to which they had randomly been assigned and performed the test 0, 30 min, 90 min and 150 min from drinking the beverage. They were asked to look at a white cross in the centre of the screen. When an orange dot was flashed to the right or left peripheral visual field, at variable intervals and without prompting, they were required to look in the opposite direction. Since the appearance of the stimulus evokes a reflexive movement in its direction, the task explores the ability to suppress such response by producing an antisaccade. Subjects performed the test seven times per time point. The following measures were considered: error rate, latency, gaze deviation along the Y axis and pupil diameter.
Results: Analysis of saccadic latency, *i.e.* the interval between stimulus appearance and initiation of the antisaccade, yielded a significant difference in the treated group 30 min ($p = 0.0062$) and 90 min ($p = 0.0417$) from drinking the alcoholic beverage. Pupil diameter was different in the two groups, but differences were significant ($p< 0.05$) only at 150 min. No significant ethanol effects were noted for antisaccade error rate and oscillation on the Y axis.
Conclusion: Visual reflex inhibition is impaired even by low blood alcohol levels. The Eye-tracker proved to be a valuable tool to study attention and vigilance after exposure to low alcohol concentrations.
Keywords: ethyl alcohol, eye movements, saccades

P065. *Caveat* in carbohydrate deficient transferrin determination for diagnosis of alcohol abuse: physiological increase in pregnancy

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Introduction: Carbohydrate deficient transferrin (CDT) is the collective name of a group of minor glycoforms of tansferrin (Tf), whose serum concentration increases after chronic sustained alcohol use (60-80 g per day for at least 10 days). Discovered in 1978, at present, it is considered one of the most reliable marker of chronic alcohol abuse in both clinical and forensic environment. Despite early reports on diagnostic inaccuracy in different physiological and pathological conditions (tobacco smoking, liver diseases, diabetes mellitus, treatment with anti-epileptic drugs, etc.), the most recent literature based on advanced analytical techniques (specific immunoassays, HPLC and capillary electrophoresis) proves that diagnostic specificity of CDT is extremely high, approaching 100%. Moreover, few recent works on the physiological levels in children report CDT concentration comparable to adults even in newborns. However, since protein glycosylation processes, which are at the basis of CDT formation, are affected in any physiological pregnancy, in the past a possible interference of this condition on the CDT measurement was investigated, but with methods showing limits in quantification.
Aim: The present work was a re-evaluation of the CDT pattern in pregnancy by using up-to date technology based on HPLC with detection at 460 nm.
Methods: Blood samples from pregnant women, collected during pregnancy for routine clinical chemistry controls, were unanimously analysed for CDT

determination. In detail: 44 samples were collected in the first trimester of pregnancy, 55 in the second and 42 in the third. After clotting, serum, undergone to iron saturation and lipoprotein precipitation, was analyzed by anion exchange HPLC by using a salt gradient elution (buffer A: 10 mM BIS-TRIS pH 6.2; buffer B: 10 mM BIS-TRIS+500 mM NaCl; buffer C: 2 M NaCl). The detection was based on the measurement of Tf iron complex absorbance at 460 nm. CDT value was expressed as the percentage ratio of asialo-Tf (when detectable)+disialo-Tf on the total Tf.

Results: Percentage CDT measurements in the three groups are depicted in the following table (mean percentages ± SD).

	1st trimester	2nd trimester	3rd trimester
number of samples	44	55	42
mean percentage	1.03	1.39	1.60
SD	0.20	0.27	0.24

The statistical evaluation of data (Student t test for unpaired data) showed a highly significant ($p<0.0009$) increase of CDT during pregnancy. Worth noting is that the average CDT concentrations in the third trimester are statistically higher ($p<0.0009$) than the average values measured in the general population (N: 102; males: 85; females: 17; mean: 1.31; SD: 0.33), including social drinkers, used to establish the cut-off levels (1.90%) currently adopted for diagnosis of chronic alcohol abuse.

Conclusions: The results of the present work show a potential cause of misdiagnosis of alcohol abuse in pregnancy, if based only on CDT determination. Since CDT increases become significant *versus* normal reference values only in the third trimester, when the pregnancy state is patent, interpretation errors can be easily avoided, if the phenomenon is known.

Keywords: carbohydrate deficient transferrin, pregnancy, HPLC

P066. Roadside testing for illicit drugs - survey results for 2008 year in East Bohemia region of the Czech Republic

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Introduction: Driving a motor vehicle under the influence of drugs is a dangerous phenomenon in the current traffic situation. It is one of the most important factors in traffic accidents and as it turned out, it is a problem especially for young people. For this reason, we have developed in the cooperation with metropolitan police the check system basically consisting in the effective analytical tool confirming the police procedures and opinions regarding the use of drugs of abuse by drivers.

Aim: The purpose of this contribution was to give a survey of results obtained from our common project based on the close cooperation between our laboratory and metro police during 2008 year.

Methods: A suspect driver was stopped by the police for unusual, abnormal behaviour while driving a car. A policeman from the specially trained unit carried out the determination of alcohol in breath first, and then the oral test for the presence of drugs in saliva specimen using the on-site tester. At the same time a driver was asked to provide blood and urine for confirmation testing by GC-MS and LC-MS^n in our laboratory, where we use the usual standard analytical procedures for that purpose including liquid-liquid or solid phase extraction with relevant derivatization before injection into the GC-MS.

Results: During 2008 year police officers investigated 198 (186 men and 12 women) suspect drivers with alcohol negative test results in East Bohemia territory. A median value of age for men was 23,5 years, for women was 22,5. Drugs of abuse were found in 148 (79,6%) men and 11(91,7%) women. 94(63,5%) men were positive for cannabis, 23(15,5%) for methylamphetamine, 24(16,2%) for the abuse of methylamphetamine and cannabis simultaneously, 2(1,4%) for MDMA, 4(2,7%) on a combination of cannabis and MDMA, multidrug use of MDMA and cannabis with heroin was detected in one man. Methylamphetamine was found in 8(66,7%) women. One woman was positive on a combination of methylamphetamine and cannabis and 2(16,7%) women were accused of driving under the influence of cannabis. No other drugs have been found in all provided samples.

Conclusion: We have reached a high consent between data obtained from police investigations and our confirmation results. In this way, we have verified the relatively high level of ability of some policemen to identify drugged drivers directly on the road.

Keywords: illicit drugs, roadside testing, driving under the influence, on-site devices

P067. High mortality among people suspected of drunken driving. An 18-year register-based follow-up

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Introduction: Alcohol drinking and driving under the influence (DUI) of alcohol are associated with numerous harmful health and social effects. DUI offenders are more likely to be involved with fatal road traffic accidents but there is little evidence of other excess mortality in DUI offender population.

Aims: The aim of this study was to examine overall and cause-specific mortality of DUI offenders compared to reference population with no history of DUI and to recognize risk factors of premature death.

Methods: The data used were a register of all suspected drunken drivers with between April 1988 and December 2006 (N=112,394) and their deaths (N=14,636). All drivers with drug-positive samples were excluded. DUI suspects were compared to reference population with no DUI offence previous to case. Survival analysis methods were used to study the risk factors and to compute overall and cause-specific hazard ratios.

Results: Alcohol causes, diseases of circulatory system and accidents were the most common causes of death amongst DUI suspects. DUI was linked with higher mortality in every observed group. Especially risk of death by alcohol-related or external cause was high compared to reference population. Among women the DUI apprehension increased risk of death more than among men. Within the group of DUI suspects the risk of death was affected by age, sex, marital status, education, recidivism as well as time and observed blood alcohol level of the apprehension. Half of the DUI cases and every fifth of the references had alcohol as contributing factor to death.

Conclusions: Drunken driving is a severe indicator of elevated risk of death. As premature deaths and preceding health harms are costly to society these deaths should be prevented more efficiently. Several factors increasing the risk of death could be used to recognize the high risk groups for whom to target interventions to.

Keywords: driving under influence, DUI, mortality, premature deaths

P068. Comparison of drug concentrations in whole blood, plasma and oral fluid samples collected from drivers suspected of driving under the influence

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Introduction: Drugs of abuse and medicinal drugs can be analysed from different biological matrices. Blood and urine are the conventional matrices used in drug testing but their collection is quite invasive. Oral fluid (OF) is an

interesting alternative matrix because of its easy collection. It also contains the pharmacologically active drug compounds diffused from blood thus indicating that the person in question is under the influence when the sample is taken. This is very useful for example in DUI cases. In order to use OF as a reliable sample matrix more research has to be done on the correlation of drug concentrations between blood and OF.

Aim: The aim was to see if there is correlation in the drug concentrations between the three sample matrices and to calculate the blood/plasma (B/P), OF/blood (OF/B) and OF/plasma (OF/P) ratios for different drugs.

Methods: Whole blood, plasma and OF samples were collected from voluntary drivers suspected of DUI. Drugs of abuse and medicinal drugs were extracted from whole blood and plasma samples with LLE and analysed with GC–MS. OF samples collected using Statsure SalivaSampler collection device were weighed before analysis to determine the ratio of OF and buffer solution used in the device to preserve the sample. Benzodiazepines were extracted from the OF samples with LLE followed by SPE and other drugs were extracted with LLE and the analysis was done with GC–MS.

Results: From the 28 volunteers 14 cases were positive for amphetamine in all three matrices but only 12 OF sample results could be used in the calculations because in two cases the sample volume was too low to get reliable results. There was a good correlation in the amphetamine concentrations between whole blood and plasma and there was also correlation between OF and whole blood/plasma. The mean value of B/P was 1.0 and for OF/B (and OF/P) 41. Comparable results (N>5) were also found for diazepam, nordiazepam, oxazepam, temazepam, alprazolam, and clonazepam. The correlation of these benzodiazepine concentrations was good between whole blood and plasma, but only the concentrations of nordiazepam, oxazepam, and temazepam in OF showed correlation with whole blood and plasma. The number of positive findings for the benzodiazepines was lower in OF than in whole blood and plasma. The mean B/P for diazepam, nordiazepam, oxazepam, temazepam, alprazolam, and clonazepam was 0.58, 0.58, 0.64, 0.62, 0.74 and 0.65, respectively. OF/B(OF/P) for nordiazepam, oxazepam, and temazepam was 0.04(0.02), 0.23(0.14), and 0.60(0.74), respectively.

Conclusion: Although the number of amphetamine positive samples in this study is still quite small, the results indicate that any of the three matrices studied could be used to detect amphetamine from drivers suspected of DUI. For the benzodiazepines, there was some indication of correlation between OF and blood for nordiazepam, oxazepam, and temazepam but more samples are needed for reliable conclusions.

Keywords: drugs of abuse, whole blood, saliva/oral fluid, plasma, GC–MS

P069. Evaluation of the use of an enzyme immunoassay for a rapid determination of ethyl glucuronide in serum

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Introduction: Ethyl glucuronide (EtG), a minor metabolite of ethanol, is detectable in blood, urine, and hair after ethanol intake. Consequently, EtG is a sensitive and specific marker of ethanol consumption. Mass spectrometric methods, such as LC-MS/MS, have been described for the determination of EtG and are considered as reference methods. Recently, an enzyme immunoassay has been proposed for the determination of EtG in urine. This test allows rapid and sensitive screening, that can be used in order to discard negative samples.

Aims: The aim of this study was to evaluate the use of an immunoassay for a rapid determination of ethyl glucuronide in serum and to compare the results with other markers of ethanol intake (ethanol, CDT, gGT, ASAT, ALAT).

Methods: 54 serum samples, randomly selected, were obtained from patients of the Traffic Medicine Unit. Subjects, 49 men and 5 women, were Swiss drivers referred to the University center of Legal Medicine because of driving while under the alcohol influence or because of reapplying for a driving license. The age of the patients was ranging from 20 to 62 years old (mean: 40). Analysis of EtG was performed using a DRI® EtG enzyme immunoassay on a MGC240 analyzer (Thermo Fisher Scientific), with a limit of decision of 100 mg/l. Ethanol was determined by HS-GC-FID. CDT was determined by electrophoresis (Ceofix CDT reagent (Analis) on a Hewlett Packard 3D-CE) and by immunoassay (N Latex CDT kit on BNproSpec, Dade-Behring Siemens). The following enzyme activities were measured in serum : gGT, ASAT and ALAT (Dimension, Dade Behring Siemens).

Results: EtG was detected in 11 cases (mean: 1680 mg/l, minimum: 129 mg/l, maximum: 4650 mg/l). The subjects with serum positive EtG were only men. EtG was detected in all samples where ethanol concentration was higher than 0.1 g/kg (N=4). When EtG was not detected, ethanol was not observed (N=43). In seven cases EtG was observed and ethanol concentration was lower than 0.1 g/kg. The presence of EtG in serum (N=11) was correlated with high values of CDT (CE) (mean: 5.1%). In comparison, the absence of EtG in serum (N=43) was correlated with lower values of CDT (CE) (mean: 2.2%). Similar results were obtained for CDT (NLatexCDT) (mean: 4.9% vs 2.8%), gGT (mean: 131 U/L vs 81 U/L), ASAT (mean: 40 U/L vs 24 U/L) and ALAT (mean: 62 U/L vs 47 U/L).

Conclusion: A rapid enzyme immunoassay can be used as screening test for the determination of ethyl glucuronide in serum. Nevertheless, positive EtG immunoassay results must be confirmed by mass spectrometric methods, such as LC-MS/MS.

Keywords: Ethyl glucuronide, Enzyme immunoassay, Alcohol

Drugs of abuse

P070. Effects of opium addiction on some serum factors of alloxan-induced diabetic rats

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Introduction: Traditional opium was given orally (10 mg / kg body weight) to all experimental rats except the control negative (normal health) group for 30 days.

Aim: The purpose of our work was the study was carried out to determine the effect of opium on iochemical parameters in addicted rats.

Methods: Diabetes mellitus was induced in adult male albino rats, using intra- peritoneal injection of 120 mg / kg BW. Blood glucose,serum insulin, total protein, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TGs) and total cholesterol were measured in the serum of rats.

Results: Serum total protein, ALT and AST were lower compared to non-addicted diabetic rats. Cholesterol and triglycerides tend to be lower in addicted diabetic rats. Creatinine and urea were higher in addicted diabetic rats compared to non-addicted diabetic rats. According to our results, opium increases serum insulin and decreases serum glucose but non-significantly, and thus adds to metabolic disorders in diabetic rats.

Conclusion: These results suggest that opium reduces blood glucose in diabetice rates and the mechanism of this effect is unclear

Keywords: opium, alloxan.

P071. Validation of a detection level of 25 ng/mL for cannabinoids in urine, using a Cedia THC PLUS reagent. Application of this cut-off to urines of school children

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Introduction: Marijuana is the most widely used illicit drug in New Zealand. Recent longitudinal studies have shown that adolescent abuse of cannabis may not only develop dependence but also impair life achievements (1).

Many drug prevention programmes take place in school settings and one of them is testing for illicit drug abuse. Detection and intervention of cannabis abuse at an initial stage of a child's life can avoid the progression of such abuse. Because some of the school principals of the schools that were undertaking drug prevention programmes here at New Zealand, suspected comparatively low cannabis use, it was pertinent to investigate the detection at concentrations substantially less than the presently accepted cut-off of 50 ng/mL.

Aim: LabPlus is accredited AS/NZ4308 to do evidential drug testing for employment and legal purposes. The objective of this study was to validate having two different urinary cut-off values for cannabinoids: 50 ng/mL for evidential drug testing and 25 ng/mL for clinical, drug abstinence programmes and school drug testing programmes.

Methods: 3119 human urine samples were collected between June and August and between October and November 2008. They were screened by immunoassay, for the presence of cannabinoids using the CEDIA® THC PLUS (ThermoFisher, Microgenics products) reagent. Specimens with values of 20-40 ng/mL were subjected to an additional immunoassay procedure using a 25 ng/mL calibrator in order to increase the sensitivity within that range. 695 (22.3%) were of persons aged less than 18 years (school aged children). Samples that had an immunoassay value between 13 to 49 ng/mL were further subjected to confirmatory testing by gas chromatography-mass spectrometry (GC-MS) for the presence of 11-nor-9-carboxytetrahydrocannabinol (THCCOOH). The lower limit of confirmation by GC/MS is 4 ng/m.

Results: The total number of positives reported at the screening cut-off of 50 ng/mL was 888 (28.5%). However, having a screening cut-off at 25 ng/mL increased the number of positives to 1059 (34.0%).

Of the 171 samples that had immunoassay values between 25 to 49 ng/mL, 108 samples were available, and underwent confirmation testing by GC/MS for the presence of THCCOOH. In all the 108 samples the presence of THCCOOH was confirmed. Another 64 urine samples that had a cannabinoid value between 13 to 24 ng/mL were similarly confirmed by GC/MS. This demonstrated that 25 ng/mL is well above the detection threshold of the Cedia screening immunoassay.Similarly, concerning the school aged children, 34.6% screened positive at the 50 ng/mL cut-off and this increased to 41.4% at the 25 ng/mL cut-off.

Conclusion: In the present study, utilizing a cut-off of 25 ng/mL rather than 50 ng/mL, we demonstrated that a considerable number of false negative results were averted. For the school aged children, utilizing the 25 ng/mL cut-off, the sensitivity is improved by detecting 7% more of those on cannabis. For these children appropriate intervention and preventive measures may not have therefore occurred if using the higher cut-off value of 50 ng/mL.

Reference: [1] Cannabis use and later life outcomes; A longitudinal study of a New Zealand birth cohort studied to age 25 years. Fergusson DM & Boden JM.; Addiction; 2008; 103: 969-976.

Keywords: cannabinoids, urine, cut-off levels

P072. Methylecgonidine and ecgonidine detected as pyrolytic artifacts in GC-MS analysis of cocaine positive specimens

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Introduction: When free-base cocaine is smoked, methylecgonidine (MED) is formed as a pyrolytic compound, and consumed with cocaine. Therefore, detection of MED and its metabolite ecgonidine (ED) in urine are being used as markers to differentiate cocaine smoking from other routes of administration. But it has been reported that MED can be a pyrolytic artifact of cocaine and ecgonine methyl ester (EME) when tested by GC-MS with elevated temperatures. Similarly, ED could also be a pyrolytic artifact of benzoylecgonine (BZ) and ecgonine (EC). While GC temperature is the major cause of artifact production, the urine matrix may also contribute to the formation of the compounds. In this study we examined the effects of urine pH and specific gravity on the formation of the artifacts.

Methods: Twenty different negative urine specimens collected from a random urine drug screening program were tested for pH and specific gravity. The specimens were spiked with a fixed amount of cocaine, EME, BZ, and EC at concentrations 3 µg/mL, 15 µg/mL, 16 µg/mL, and 3 µg/mL, respectively. The concentrations are representative of those in a human study we had completed in recent years in which MED and ED were detected in non-smoking routes (unpublished). The samples were extracted and analyzed according to a published method [Biomed Chromatogr, 2005; 19: 677-688]. In the two-stage solid-phase extraction, COC, MED, BZ, and EME were separated from ED and EC. While the MED was tested with the pyrolytic precursors, COC and EME, the ED was tested with the pyrolytic precursor EC, but was free from the other precursor, BZ. ED was tested as a pentyl derivative. Three ions for the drugs and two ions for the deuterated internal standards were used in the GC-MS analysis.

Results: The injection port temperature in the GC was kept at 140 ^{0}C to minimize artifact formation. MED was detected in all specimens (Mean $\pm$ SD = 9.3 $\pm$ 1.6, Median = 9.3, Max = 12.8, Min = 6.7 ng/mL). When the specimens were re-injected without changing any injection port conditions, the amount of MED increased (Mean $\pm$ SD = 11.3 $\pm$ 2.7, Median = 11.8, Max = 17.5, Min = 7.4 ng/mL). The means are significantly different (Students paired $t = 6.02$, $p < 0.001$) indicating that deposits in the injection port contributed to the artifact formation. This type of injection port effect is similar to the effect that leads to the formation of methamphetamine from ephedrine and pseudoephedrine. The highest concentrations of MED were found when the urine pH was in the range of 5.1 to 5.8 and specific gravity in the range of 1.0154 to 1.0318. These types of matrices appeared to contribute maximum deposits in the injection port and increase artifact formation. There were ED peaks in five specimens, but in all cases the ion ratios were outside $\pm$20% (concentration range 1.8 to 2.3 ng/mL). Therefore, separation of BZ from ED with the two-phase extraction minimized the artifact formation. In all cases the amounts of both MED and ED were less than 0.6% of any artifact precursor.

Conclusion: The urine matrix appeared to influence the artifact formation of MED and ED from cocaine compounds. In all cases the amounts were <20 ng/mL for MED and <10 ng/mL for ED. The detection of MED and ED are still considered useful for determination of cocaine ingestion by smoking when the GC-MS analyses are performed under controlled conditions. In addition, the use of an artifact control that contains pyrolytic precursors is recommended in each batch analysis.

Keywords: cocaine smoking, methylecgonidine and ecgonidine, analytical artifacts

P073. On-line solid phase extraction system combined with liquid chromatography-tandem mass spectrometry for high throughput analysis of 11-nor-δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine

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Introduction: One of the bottlenecks in bioanalysis is often associated with the sample preparation requirements. A very elegant system for rapid analysis of complex samples can be obtained by the on-line coupling of SPE to LC-MS detection.

Aims: The development and validation of a simple, rapid and highly sensitive method for the analysis of THC-COOH in urine using an automated on-line solid phase extraction (SPE) system (Symbiosis Pharma, Spark Holland) combined with liquid chromatography (LC)-mass spectrometry (MS/MS).
Methods: Chromatographic separation was achieved using a Atlantis dC_{18} column and an isocratically elution with acetonitrile:0.1% formic acid (80:20, v/v). Just 500 µL of urine was required. On-line SPE was carried with C_8 cartridges, using the mobile phase as elution solvent. Selectivity of the method was achieved by a combination of retention time and two precursor-product ion transitions for the non-deuterated compound in ESI positive mode (345.2>299.3, 345.2>193.1).
Results: The elution of THC-COOH was ensured within 4.1 min. The total process time was 6 minutes. The use of SPE using C_8 cartridges was demonstrated to be highly effective and led to significant decreases in the interferences present in the matrix. Extraction was found to be both reproducible and efficient. The method showed an excellent intra-assay and inter-assay precision (relative standard deviation (RSD) <7% and bias <13%) for four external quality control (QC) samples and three 'in house' QC, and the r^2> 0.99 over the range investigated (5-200 µg/L). Limits of quantification (LOQ) and detection (LOD) were estimated to be 5 µg/L and 0.25 µg/L, respectively. Furthermore, the hydrolyzed samples kept in the autosampler were demonstrated to be stable for at least 24 hours. The method was subsequently applied to authentic samples previously screened by a routine immunoassay method.
Conclusion: The method combined on-line SPE with LC-MS/MS and provided a thorough clean-up of the matrix in combination with high recovery, excellent precision and accuracy in the linear range investigated. The method was successfully applied to authentic samples from cannabis users. This method is certainly of interest on the field of forensic toxicology.
Keywords: on-line SPE, cannabis, LC-MS/MS, urine

P074. Simultaneous determination of methamphetamine, 3,4-methylenedioxy-N-methylamphetamine, 3,4-methylenedioxy-N-ethylamphetamine, N,N-dimethylamphetamine, and their metabolites in urine by liquid chromatography-electrospray ionization-tandem mass spectrometry

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Introduction A liquid chromatography-electrospray ionization-tandem mass spectrometric (LC-ESI-MS/MS) method was developed and validated for the simultaneous detection and quantification of seven amphetamine derivatives (amphetamine (AP), methamphetamine (MA), 3,4-methylenedioxy-N-amphetamine (MDA), 3,4-methylenedioxy-N-methamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), N,N-dimethylamphetamine (DMA) and N,N-dimethylamphetamine-N-oxide (DMANO)) in human urine. Especially, the N-methylated compound of MA, DMA, has recently been used illegally in Korea.
Aims The sensitive LC-ESI-MS/MS method using automated solid-phase extraction (SPE) and a narrow-bore HPLC column was developed for simultaneous determination of the most frequently abused drugs and metabolites, such as amphetamine, methamphetamine, methylenedioxy derivatives, DMA and DMANO.
Methods Seven deuterium-labeled compounds were prepared for use as internal standards to quantify the analytes. One milliliter of urine was combined with 1 mL of 0.2 M carbonate buffer solution (pH 9.0) before solid phase extraction (SPE). An Oasis HLB SPE column followed by chromatographic separation on a Capcell Pak C18 MG-II column (150 × 2.0 mm I.D., 5 µm) and electrospray mass spectrometry with multiple reaction monitoring were used for selective and sensitive detection. The use of ammonium formate (5 mM, pH adjusted to 4.0 with formic acid, Solvent A) and acetonitrile (Solvent B) as the mobile phase at a flow rate of 230 µL/min was found to be the most effective for the separation.
Results: The linear ranges were 5.0-1000 ng/mL for AP, MDA, MDMA, MDEA, DMA and DMANO and 10.0-1000 ng/mL for MA, with good correlation coefficients (r2 > 0.996). The intra-day, inter-day and inter-person precisions were within 14.6%, 12.1% and 15.5%, respectively. The intra-day, inter-day and inter-person accuracies were between -11.6 and 9.0%, -7.9 and 2.3%, and -13.2 and 4.3%, respectively. The limits of detection (LODs) for each analytical compound were lower than 1.95 ng/mL. The recovery ranged from 72.3 to 103.3%.
Conclusions: This method is a sensitive and reliable LC-MS/MS method for the simultaneous detection of amphetamine-type stimulants in human urine. The SPE step was assayed to detect and quantify seven target analytes in urine samples without any significant interference from the matrix components. The applicability of the developed method was examined by analyzing several urine samples from confirmed drug abusers.
Keywords: LC/MS, analysis of amphetamine-type stimulants in urine

P077. Urinary concentrations of buprenorphine and its metabolites in patients taking buprenorphine

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Introduction: Urinary buprenorphine (Subutex, Suboxone) assay is useful in monitoring buprenorphine therapy. A 'cut-off' of 4 µg/L has been suggested for total buprenorphine by GC-MS, *i.e.* after hydrolysis of buprenorphine glucuronide. Commercial buprenorphine immunoassays vary in cross-reactivity with buprenorphine itself and with buprenorphine metabolites. The Microgenics Buprenorphine Assay (CEDIA) uses a cut-off of 5 µg/L and may give false negative results in clients undergoing opioid detoxification.
Aim: To investigate the clinical relevance of the 5 µg/L cut-off in the Microgenics Buprenorphine Assay with respect to typical buprenorphine opioid detoxification regimens.
Methods: Urine samples from buprenorphine-treated clients were analysed by (i) Microgenics CEDIA Buprenorphine Assay, (ii) before and (iii) after enzymatic hydrolysis (ß-glucuronidase, 60 °C, 4 h) by LC-APCI-MS/MS (TSQ Quantum Access, Thermo Scientific). APCI conditions: corona needle discharge current 5 µA; temperatures: vaporiser 350 ºC, capillary 225 ºC; sheath gas 60 (arbitrary units); positive ion mode. For LC-MS/MS, samples (3.0 mL) and deuterated internal standards (100 µg/L buprenorphine-D_4 and norbuprenorphine-D_3, 50 µL) were loaded onto pre-conditioned solid phase extraction cartridges (Phenomenex Strata Screen-C), washed with acetic acid (1 mol/L, 1 mL), deionised water (2 mL) and methanol (2 mL), and eluted with 2 mL dichloromethane:2-propanol (80+20) with 2% (v/v) ammonium hydroxide solution (25% w/v). The extracts were evaporated (compressed air, 40 ˚C), reconstituted in methanol and analysed on a 100 × 2.1 mm i.d. Waters Spherisorb S5SCX column with methanolic ammonium acetate (40 mmol/L, pH* 6.0) as eluent. Retention times of buprenorphine and norbuprenorphine were 1.99 and 2.68 min, respectively. Buprenorphine and norbuprenorphine were measured by monitoring surviving pseudomolecular ions (m/z 468 and 414, respectively). Qualifier ion transitions (*m/z*) were: buprenorphine (468 → 396, 414), norbuprenorphine (414 → 225, 101). Assay calibration (range 2-100 µg/L both analytes, r^2>0.99) was by peak height ratio to the respective internal standards. The limits of detection were 0.1 and 0.2 µg/L for buprenorphine and norbuprenorphine, respectively.
Results: Of 100 urine samples from clients prescribed buprenorphine analysed by CEDIA (median buprenorphine 47.0 µg/L, range 2.6–131.3 µg/L), only 12 had pre-hydrolysis buprenorphine concentrations greater than the stated

5 µg/L cut-off (median 8.6 µg/L, range 5.3–46.5 µg/L) when analysed by LC-MS/MS. Samples (n = 10) from patients undergoing buprenorphine detoxification, which gave low buprenorphine concentrations by CEDIA (2–15 µg/L), produced comparable post-hydrolysis buprenorphine concentrations by LC-MS/MS. The same samples were all 'positive' for post-hydrolysis norbuprenorphine, (5 µg/L cut-off; median 34.5 µg/L, range 9.4–74.0 µg/L).

Conclusions: Buprenorphine is primarily excreted in urine as buprenorphine-3-ß-D-glucuronide, free norbuprenorphine, and norbuprenorphine-3-ß-D-glucuronide. CEDIA has minimal cross-reactivity with norbuprenorphine and its conjugates, and can produce false negative results for clients undergoing detoxification. Assay of norbuprenorphine is more useful in these instances. Norbuprenorphine has a longer plasma half-life, and is present in larger amounts in urine, than the parent compound, and thus urinary norbuprenorphine assay gives a more sensitive indication of buprenorphine use.

Keywords: buprenorphine, norbuprenorphine, urine, LC-MS/MS, CEDIA

P078. Growing impact of drug misuse on emergency departments

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Introduction: Emergency drug screening is performed by specialist laboratories to aid in the diagnosis and treatment of potentially poisoned patients. This is because specialist analytical toxicology services are acknowledged to reduce the average length of stay of patients in hospitals, especially in complicated cases. The types of drugs frequently encountered are the commonly prescribed sedative tranquilizers such as antidepressants, pain relief agents and alcohol. However periodic review of the data has suggested that there has been an increasing trend in the detection of illicit drug use in the presenting patient population.

Aims: The aim of this study was to review the results generated by the analytical services provided by The West Midlands Toxicology Laboratory to determine the extent of the involvement of illicit drug use in patients being admitted to hospital for emergency treatment. The data was also used to determine if any trends in illicit drug misuse could be discerned which may impact on the requirements for future analytical service provision.

Methods: Emergency drug screening (EDS) is performed using a variety of complimentary techniques including illicit drugs monitoring using an Olympus AU640 automated platform utilizing Microgenics CEDIA® reagents, in-house GC techniques for alcohols and a range of commonly prescribed sedative compounds, together with HPLC and GCMS techniques as required for specific drugs. Illicit drug use detected following EDS was collated between January 2000 and December 2008 and the findings have been compared to illicit drug use data from drug dependency units.

Results: Data from nearly 6000 EDS cases was collated. Between 2000 to 2008 large increases in amphetamine (40%), cocaine (132%), methadone (92%) and opiates (29%) were noted for male patients. Although there was a negative trend for amphetamine (-21%) in female patients, data for cocaine, methadone and opiates increased by 274%, 767% and 127% respectively. Apart from cocaine, increases in the prevalence of illicit drug use in the emergency setting are far greater than in drug dependency units suggesting there may be two distinct drug misuse populations.

Conclusions: The detection of illicit drug use in the emergency setting has been demonstrated to be a growing problem for the clinical staff and the analytical support services. However, it must be noted that although illicit drug use is likely to be the reason behind many emergency hospital admissions, the detection of illicit drug use could also merely reflect lifestyle choices. It is therefore vital to asses drug involvement in each case on an individual basis.

P079. Monitoring naltrexone to support opiate detoxification programs

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Introduction: Naltrexone was first synthesized in 1965 and tested during the 1970s and 1980s as a pharmacotherapy to support opiate dependent individuals. Although naltrexone was found to be a potent and effective medication for the treatment of opioid dependence, it is not routinely used in clinical practice, and is only prescribed to between 1% and 3% of opioid-addicted people who might benefit from its use. However, lack of adherence to detoxification programs and limited retention in treatment have long been associated with naltrexone therapy.

Aims: The aim of this study was to develop a quantitative GCMS method which could be used to measure the concentration of naltrexone and its primary metabolite 6β-naltrexol in urine and plasma following routine use. It was hoped that the method could be used to optimise treatment. The validity of this approach was tested in an individual being treated with naltrexone but who had continuing opiate craving and abuse.

Methods: All samples were first hydrolysed using β-glucuronidase. Following the addition of nalorphine as the internal standard, specimens were extracted at pH 9 using chloroform:isoporpanol 9:1 (v:v). The solvent layer was evaporated to dryness and then initially derivatised using methoxylamine and subsequently by BSTFA to form TMS derivatives. The resultant extract was then transferred into an autosampler vial for analysis. All analyses were performed on an Agilent HP 6890 series GC incorporating an Agilent HP5973 MSD operated in SIM mode.

Results: The assay was found to be linear over the range 1 ng/mL to 50 ng/mL ($r^2 > 0.997$), with a limit of quantitation of 1 ng/mL in plasma and 15 ng/mL in urine. The within and between batch assay reproducibility was found to be less than 13% with a between batch accuracy of > 90%. The mean plasma naltrexone and 6β-naltrexol concentrations were found to be 1.1 ng/mL (range 1.0 ng/mL to 1.4 ng/mL) and 4.1 ng/mL (range 3.2 ng/mL to 4.9 ng/mL) respectively.

Conclusions: The application of this technique determined that the concentrations of naltrexone in this individual were between 45% and 75% lower than the target concentration stated by the manufacturer and were insufficient to block euphoric effects of a heroin challenge. It is hoped that the application of this method may help in the diagnosis of naltrexone treatment failures by explaining any potential therapeutic failures in opiate dependent individuals.

P080. Comparison of extraction methods for screening of drugs and drugs of abuse in human urine samples using GC-MS

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Introduction: Screening for drugs and drugs of abuse in biological samples is very important in clinical and forensic toxicology. Urine is used as primary specimen in screening analyses of unknown drugs or poisons, owing to the higher concentrations and longer detection windows of compounds of analytical interest and their metabolites, compared to blood.

Aim: The object of the study was to compare liquid-liquid extraction (LLE) and solid phase extraction (SPE) techniques for screening for commonly encountered drugs and drugs of abuse in spiked drug-free human urine samples using gas chromatography-mass spectrometry (GC-MS).

Methods: A mixture of codeine, morphine, ephedrine, 3,4-methylene-dioxymethamphetamine, tramadol, dothiepin, cocaine, mirtazapine, clomipramine, alprazolam, zolpidem, clozapine, amitriptyline, citalopram, diazepam, levomepromazine, bromazepam, phenobarbital, and guaifenesin was chosen for the evaluation of the extraction methods. After addition of internal standards trimipramine-d_3 for basic analytes and hexobarbital for acidic analytes, respectively, 2 ml of drug-free human urine were spiked with tested mixture of standards and extracted: 1) LLE technique: basic and neutral analytes were extracted with 4 mL of ethylacetate:1-chlorbutane:cyclohexane (3:1:1 v/v/v) (pH 8.0-9.0) and acidic and neutral analytes were extracted with 4 mL of ethylacetate:toluene (4:1 v/v/v) (pH 2.0-3.0) 2) SPE technique (Bond Elute Certify cartridges, Varian): with mixed-mode columns basic and neutral drugs were eluted with dichlormethane:isopropanol:ammonia (8:2:0.2 v/v/v) and acidic and neutral drugs were eluted with acetone:dichormethane (1:1 v/v). Analytes were separated on HP-5 ms 30 m × 0.25 mm i.d. with 0.25 μm film thickness. The compounds were screened for and identified using a Finnigan MAT MAGNUM ion trap GC-MS with Varian 3400 GC fitted with SPI injector and A200S autosampler operated in full scan mode. In the case of process of real samples from clinical practices, an acid hydrolysis was used to cleave metabolic conjugates. After acid hydrolysis of one aliquot of urine, another aliquot of native urine was added and the mixture was liquid-liquid or solid phase extracted, silylated and GC-MS analyzed.

Results: Studied extraction techniques were evaluated by the extraction recovery assessment. LLE showed extraction efficiency ranging from 71.2 to 96.8 and SPE from 64.6 to 107.1%. The evaluated calibration curves were linear for all drugs and both extraction techniques covering the concentration range 50 – 2000 ng/mL with calibration coefficients above 0.997 for LLE and 0.998 for SPE. The limit of quantitation was established as 50 ng/mL and limit of detection below this level for both LLE and SPE techniques, respectively.

Conclusion: Based on our experiments, LLE is more successful method for universality, while SPE is more useful method for selectivity. The application of the described assay was tested by real samples analysis.

Keywords: extraction techniques, screening, drugs, gas chromatography-mass spectrometry

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P081. A sensitive and validated GC/EI-MS method for the determination of δ^9-tetrahydrocannabinol and its metabolite 11-nor-δ^9-tetrahydrocannabinol-9-carboxylic acid in whole blood

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Introduction: Cannabis is a commonly abused drug in Greece and is often implicated in driving control or accidents and forensic cases. The determination of Δ^9-tetrahydrocannabinol (THC) and its metabolite 11-nor- Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) in blood is a necessity for a toxicological laboratory.

Aims: The aim of this study was the development of a sensitive, specific and accurate gas chromatography-mass spectrometric method, providing improved full validation data, for the determination of THC and THCCOOH in whole blood. In cases involving drugs and driving, identifying of THC in blood provides unequivocal proof that a person was under the influence of cannabinoids. Furthermore, determination of low blood concentrations of THC is required because of THC's short half life time.

Methods: The method procedure included solid phase extraction, using Bond Elut Certify II (Varian). This assay used THC-d3 and THCCOOH-d3 as internal standard for the determination of the corresponding analytes. SPE columns were conditioned with methanol followed by mixture of acetate buffer pH=7.0 and methanol (95:5, v/v). The samples were diluted with the above mentioned mixture. THC and THCCOOH were eluted with freshly prepared mixture of hexane:ethyl acetate (90:10, v/v) and hexane:ethyl acetate:acetic acid (90:10:1, v/v/v), respectively. The separate cannabinoid fractions were derivatized with *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) for 30 min at 70 °C, achieving minimal matrix effect and absolute recovery higher than 92.0% for each analyte. Analysis was carried out by gas chromatography electron impact mass spectrometry (GC/EI-MS) with a HP-5-MS fused silica column using selected ion monitoring.

Results: Limits of detection and quantification for the analytes were 0.7 and 2.0 ng/mL, respectively. The calibration curves were linear within the range of 2.00-200 ng/mL (R^2>0.996) for both analytes. Intra/interday accuracy and precision were found to be less than 8.53% and 4.79%, respectively, for all analytes at three quality control levels. Stability studies for THC and THCCOOH in whole blood, at room temperature for at least 2 hours, at 4 °C for two weeks and at -20 °C for one as well as three months, were also performed.

Conclusions: The method described was employed in the routine of the laboratory and was applied to whole blood samples related with forensic cases. The detection of THC in blood is important in human performance and *post mortem* toxicology in order to correlate recent drug use with impaired performance or behavior.

Keywords: cannabinoids, whole blood, GC/MS

P082. Comparison between urine and hair analysis of methamphetamine in Yaba abuse

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Introduction: Methamphetamine (MA), the main illicit substance in "yaba", is still an important drug in Thailand. Determination of MA use is usually based on urinalysis. Hair analysis is another method of choice, especially for diagnosing long-term use. In general, hair can be used to identify MA users better than urinalysis. However, this may depend on the time of last use and the amount of drug use. Not so many studies have reported the detection rates of urine and hair MA analysis in subjects who have admitted using the drug.

Aim: The purpose of this study was to determine the rate of MA detection and to compare degree of agreement between urine and hair analyses in MA abusers.

Methods: Hair and urine specimens, together with history of drug use, were collected from 502 young Thai adults who had admitted using yaba at least 3 times during the past 3 months. The method validation for MA hair analysis was reported previously. The lower limit of quantitation was 0.5 ng/mg of hair and the limit of detection was 0.3 ng/mg of hair. The cutoff level used in this study was 0.5 ng/mg of hair. After subjected to HCl extraction, hair was analyzed for MA by head space-solid phase micro-extraction (HS-SPME) coupled with a GC-MS. Urine was screened for MA with a REMEDi and confirmed by thin layer chromatography and GC-MS. MA detection rates in urine and hair were compared using Chi-square test. The agreement between urine and hair analysis was measured by kappa statistic.

Results: Seventy-five and a half percent of the subjects were male. Most of them were aged 18-25 years old in both sexes. The average history of yaba abuse was 4.94±2.40 (mean±SD) years in males and 3.58±2.11 years in females. The mean time since last yaba use was 15.12±16.14 (mean±SD) days in males and 15.40±16.34 days in females. MA detection rate in hair was 34.3% of all the subjects compared to 19.1% in urine ($p<0.001$ McNemar Chi-square test). Most of the urine MA detection was in the group that had last used yaba within 30 days, whilst 34.6% of this group showed positive MA in hair. Fifteen out of 55 subjects (27.3%) who admitted last yaba use between

30-60 days had hair MA positive. Seven out of 14 cases (50%) that last used yaba between 60-90 days showed positive MA in hair. The ratio of hair and urine MA positive was less than 1, if the last drug use was less than 7 days. MA hair analysis was detected in about 35% and 34% of the subjects that had last used yaba within 14 and 30 days, respectively. Using the admission of drug use as a gold standard, under-detection was observed in both urine and hair analysis. However, if both specimens were tested for MA, the rate of detection was over 50% of drug abusers if the last use was within 14 days. In the group that had last used yaba within 30 days, 30.5% of the cases who had used less than 3 yaba tablets per month had hair MA positive compared to only 14.3% MA positive in urine. The percent of urine detection increased to 45% for subjects who admitted using more than 30 tablets per month. There was no strong agreement between hair and urine results in the group that last used yaba within 30 days (kappa = 0.131; 95%CI = 0.022-0.240).

Conclusion: In the group that admitted drug use, both urine and hair analysis still under-detected drug use. The detection rate of urine MA depended on the last drug use. If the last use was within 7 days, it showed a higher rate of detection than hair analysis. Hair was a sample of choice for MA detection if the last yaba use was more than one week. Using both specimens will increase the chance of MA detection. There was no good agreement between urine and hair analysis for MA in these yaba abusers.

P083. Determination of gamma-hydroxybutyric acid (GHB) and its precursors in biological fluids and in street samples using solid phase micro-extraction (SPME) and liquid chromatography mass spectrometry (LC/MS)

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Introduction: The forensic investigation of a sex crime is one of the most important duties for the medical investigator. In cases where psychoactive substances are used, it is likely that no physical evidence on the body will be found. Under these circumstances the detection of the psychoactive substances in biological fluids is very important. GHB appears in physiological level in the human body and it is a metabolic product of 1,4 butanediol (1,4 BD) and gamma butyrolactone (GBL). Simultaneously, GHB is also a drug of abuse which is abused by athletes due to its positive feedback on the growing hormone. It is also associated with many sex crimes since it causes not only a sense of euphoria, but also amnesia and a rise of sexual aggressiveness. The presence of GHB, 1,4 BD and GBL in biological fluids as well as in street samples, constitutes very important information for the investigation of such cases.

Aim and methods: The purpose of this study was to develop a simple, fast and sensitive method for the detection of GHB and its precursors in whole blood and street samples using liquid chromatography mass spectrometry (LC/MS) in SIM mode, while GHB-D_6 and GBL-D_6 were used as internal standards. The technique of solid phase micro extraction (SPME) was used for the extraction of these substances from blood. This particular technique is superior to any other extraction technique using organic solvents, high cost materials. It is simple to use, fibers are reusable and provides much cleaner extracts. Two kinds of fibers where used (100 μm PDMS, 65 μm PDMS/DVB) and conditions such as absorption and desorption time, absorption temperature on the fiber and desorption temperature from it, composition of desorption and absorption solvent, ionic strength, kind of fiber etc. Chromatographic separation was achieved on Xterra MS C18 analytical column (100 × 2, 1 mm, I.D. 3, 5 μm) using different kinds of buffer solution and organic modifiers in both positive and negative mode in order to obtain the optimum response. The monitored ions for the selected positive mode were 105 m/z and 87 m/z for GHB, 91 m/z and 73 m/z for 1,4 BD, 87 m/z and 45 m/z for GBL.

Results: The method is at the point of optimization and will be fully validated and will be used for the investigation of forensic cases by our laboratory.

Conclusion: The development of this method will allow the simple and rapid determination of GHB and its precursors in whole blood and street samples avoiding laborious extraction procedures.

Keywords: GHB, LC/MS, blood

P084. Multicenter evaluation of ONLINE DAT® amphetamines II assay on Roche/Hitachi and Cobas systems

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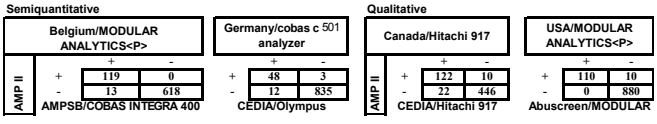
Aims: The goal of the study was to evaluate analytical performance of ONLINE DAT® Amphetamines II assay for determination of amphetamines, methamphetamines, and MDMA under routine conditions. Imprecision and agreement with routine immunoassays and reference methods were tested according to a standardized protocol in four laboratories.

Methods: Roche turbidimetric immunoassay is based on kinetic interaction of microparticles in solution (KIMS). Results using 500 ng/mL cutoff (Cobas® 6000 system, Hitachi 917, MODULAR ANALYTICS) are shown with first generation Roche Abuscreen OnLine Amphetamines, CEDIA Amphetamines/Ecstasy, or COBAS INTEGRA Amphetamines/MDMA Sensitive assays as the comparison methods. Routine drug-of-abuse screening urine samples were used in all laboratories for method comparison. Discrepant samples were identified and analyzed by an LC-MS/MS or GC/MS method.

Results: Intra-assay imprecision (21 replicates per run; 5 runs):

Site/Analyzer	Mode	Samples (ng/mL Amphetamine)	Performance (%CV)
Belgium/MODULAR *ANALYTICS* <P> and Germany/cobas c501	Semiquantitative	375 500 625	4.9-8.0 2.8-6.6 2.5-6.5
Canada/Hitachi 917 and USA/MODULAR *ANALYTICS* <P>	Qualitative*	375 500 625	0.7-1.4 0.8-1.3 0.8-1.6

*Calculated based on absorbances.



Seventy samples were discrepant and confirmed. Of the 23 samples positive with Amphetamines II, but negative by other immunoassay, 9 were confirmed positive. Of the 47 samples negative with Amphetamines II, but positive by other immunoassay, 46 were confirmed negative.

Conclusion: Results prove the reliability of Roche ONLINE DAT® Amphetamines II assay, which yields fewer false positive and false negative results than other immunoassays tested and can be recommended for routine drugs-of-abuse screening for amphetamines and related compounds on clinical chemistry analyzers.

Keywords: Amphetamines, methamphetamines, MDMA, immunoassay, urinanalysi

P085. On line sample extraction technique *vs* traditional sample preparation methods for LC-MS forensic toxicology screening

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Novel Aspect: Evaluation and comparison of three sample preparation methods implemented in forensic screening application on ion trap mass spectrometer

Introduction and Objective: LC-MS is a powerful technique for forensic drug screening. New, sensitive MS/MS systems enable detection of drugs at low levels. However the quality of LC-MS data collected in screening application is largely affected by sample preparation method. Three different urine sample preparation methods implemented in forensic toxicology screening were evaluated on set of 300 basic, neutral and acidic compounds.

Method: We compared 3 sample preparation methods to find the most robust, easy to use and efficient method to acquire quality MS/MS data. Solid phase extraction, liquid-liquid extraction and on line extraction methods were compared. SPE method for basic, neutral and acidic compounds implementing cation exchange, non-polar cartridge was developed in house. Commercially available ToxiTube product was used to evaluate liquid-liquid extraction. TurboFlow method on Aria TLX system using dual columns was developed in house.

Validation: Urine samples spiked to concentrations 1-100 ng/mL with 10 randomly selected compounds were processed with SPE, LLE and on line extraction. Processed samples were then analyzed with LC-MS screening method implemented on Quantum LXQ mass spectrometer. Compounds were identified based on MS2 spectra and retention time. In addition diluted urine samples were analyzed for data comparison.

Results and Conclusion: All evaluated sample preparation methods allowed for identification limit required in forensic toxicology screening. SPE requires longest time but it can be automated for better efficacy. ToxiTube method gives similar results to SPE but it is more efficient. On line extraction is the most efficient and most cost effective method. The on line methods can also be multiplexed to double or quadrupole sample throughput.

P086. Performance evaluation of three LC-MS methods implemented on Ion Trap Mass Spectrometer for drug testing in urine

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Novel Aspect: Evaluation of advantages and limitations of three compounds identification methods implemented on ion trap mass spectrometer.

Introduction and Objective: Drug identification in complex samples is one of most important applications in forensic toxicology laboratories. Three different methods identifying compounds based on MS2, MS3 and MS2/MS3 spectra and retention time were evaluated for drug testing in urine samples.

Method: Three methods collecting MS2, MS3 and MS2/MS3 spectra for compounds identification were developed on LXQ Ion Trap mass spectrometer. All methods included a scan depended experiment in polarity switch mode and a 13 minutes LC gradient using 50 × 2.1 mm PFP column. Spectra were collected in compound specific retention time windows with compound optimized collision energy.

Validation: Urine samples were spiked with randomly selected sets of compounds to specified concentrations processed with SPE procedure and analyzed. Compounds identification was done automatically by a software that compared collected spectra and retention time with the spectra in the library spectra. Performance of the method was validated based on number of compounds detected in the known samples.

Results and Conclusion: All 300 compounds in a sample could be easily identified with a method that utilized MS2 and Rt. Method based on MS3 and MS2/MS3 spectra could identify 80-100 compounds. The low threshold for data collection set up to achieve better LOD, led to identification of some endogenous compounds in the sample matrix The lower duty cycle of MS2/MS3 method decreased the number of spectra collected for target compounds resulting in lower hit rate. Methods implementing MS3 or MS2/MS3 spectra for compounds identification are less affected by interferences producing combined spectra than method identifying compounds based on MS2 spectra. The identification rate of MS3 or MS2/MS3 method can be increased by raising LOD.

P087. The buprenorphine assay V-Twin®/Viva-E® analyzers

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Background. Buprenorphine is a semi-synthetic opioid analgesic compound with a chemical structure similar to morphine. It is used as a substitution drug for opioid addiction treatment, but has a high abuse and addiction potential. A new protocol for measurement of buprenorphine has been developed on the V-Twin®/Viva-E® (Siemens) analyzers. The assay has a cutoff of 5 ng/mL. The assay kit (manufactured by Immunalysis Corporation, Pomona, CA) consists of liquid, ready-to-use reagents that will provide qualitative and semi-quantitative results. The data, presented in this study, was generated on the V-Twin analyzer. Similar performance was obtained on the Viva-E analyzer.

Methods. Precision was evaluated using the cutoff and +/- 25% controls according to CLSI EP5-A2. Recovery and linearity were studied by spiking buprenorphine into human urine at levels that span the calibration range (0-40 ng/mL). Calibration stability and on-board stability were assessed by testing the cutoff and +/- 25% controls over a 31-day period. Specimens (100) were analyzed and the results compared to those from the Roche Hitachi® 717 analyzer. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations. The effect of common interferences was assessed by spiking the interferents into human urine in the presence of buprenorphine at levels of +/- 25% of the cutoff.

Results. The qualitative repeatability CV's (rate) for the cutoff and +/- 25% controls ranged from 0.51-0.67% and the within-lab CV's ranged from 0.79-0.89%. The semi-quantitative repeatability (ng/mL) CV's ranged from 2.86-4.17% and the within-lab CV's ranged from 4.52-6.32%. The overlap distribution between the +/- 25% buprenorphine controls and the cutoff was less than 5%. The analytical sensitivity of the assay was found to be 1 ng/mL. Semi-quantitatively, the assay quantified buprenorphine-spiked samples between 2.3-40 ng/mL within +/- 20% of nominal values. The assay linearity range was between 1-36 ng/mL. At the 5 ng/mL cutoff the percent agreement of specimens between the V-Twin analyzer and Roche Hitachi 717 was 100%. The assay reagents had similar detection for buprenorphine and norbuprenorphine in urine with minimal cross-reactivity (< 1%) to the structurally related drugs, morphine, codeine, dihydrocodeine, and methadone at the 5 ng/mL cutoff. Potential interfering substances resulted in no false responses for the spiked +/- 25% controls relative to the cutoff. A minimum of 14 days calibration stability was demonstrated. The reagents are stable on-board the V-Twin analyzer for at least 30 days.

Conclusion. The new buprenorphine assay on the V-Twin/Viva-E analyzers is a suitable screening method for urine specimens at the cutoff level of 5 ng/mL for both the qualitative and semi-quantitative analyses.

** Product availability subject to meeting country specific registration and language requirements.*

P088. The oxycodone, meperidine and tramadol assays* on the V-Twin®/Viva-E® analyzers

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Background: Oxycodone, meperidine, and tramadol are members of the opiate class of drugs. They are semi-synthetic narcotic analgesics that have a high abuse and addiction potential. New protocols for measurement of these drugs have been developed on the V-Twin®/Viva-E® (Siemens) analyzers. The oxycodone assay has two cutoffs (100 and 300 ng/mL) and the cutoff for meperidine and tramadol assays is 200 ng/mL. The assay kits (manufactured by Immunalysis Corporation, Pomona, CA) consist of liquid, ready-to-use reagents that will provide qualitative and semi-quantitative results. The data presented in this study was generated on the V-Twin analyzer. Similar performance was obtained on the Viva-E analyzer.

Methods: Precision was evaluated using the cutoff and +/-25% controls according to CLSI EP5-A2. Recovery and linearity were assessed by spiking drugs into human urine at levels that span the assay range. Calibration stability was assessed by testing the cutoff and +/- 25% controls over a 31-day period. Specimens (100-110) for each assay were analyzed and the results compared to those from the Roche Hitachi® 717 analyzer. Cross-reactivity with structurally related drugs was assessed at different concentrations. The effect of common interferences with each assay was assessed by spiking the interferents into human urine in the presence of drug at levels of +/- 25% of the cutoff.

Results: With all assays the qualitative repeatability CV's (rate) for the +/- 25% controls and cutoffs ranged from 0.20-0.92% and the within-lab CV's ranged from 0.46-1.05%. The semi-quantitative repeatability (ng/mL) CV's ranged from 0.26-5.87% and the within-lab CV's ranged from 1.43-6.94%. The overlap distribution between the +/- 25% controls and the cutoffs was less than 5%. Semi-quantitatively, the assays quantified drug-spiked samples within +/- 20% of nominal values. The assay linearity range for oxycodone is between 30-900 ng/mL (300 cutoff) and 10-450 ng/mL (100 cutoff) and for meperidine and tramadol 25-900 ng/mL. The analytical sensitivity was found to be 10 ng/mL and 30 ng/mL for oxycodone 100 and 300 cutoffs, respectively. The analytical sensitivity for meperidine and tramadol was 25 ng/mL. At the cutoff level the percent agreement of specimens between the V-Twin and Roche Hitachi 717 was greater than 95% with all assays. The assays had minimal cross-reactivity with structurally related drugs. At the 300 ng/mL cutoff, oxycodone reagents had similar detection for oxycodone and oxymorphone, but minimal cross-reactivity to the structurally related drugs, morphine (1.81%), morphine-3-glucuronide (1.2%), codeine (2.2%), dihydrocodeine (4.2%) and noroxymorphone (0.3%). Potential interfering substances resulted in no false responses for the spiked +/- 25% controls relative to the cutoffs. A minimum of 14 days calibration stability was demonstrated.

Conclusion: The new oxycodone, meperidine and tramadol assays on the V-Twin/Viva-E analyzers are suitable screening methods for urine specimens in both the qualitative and semi-quantitative analyses.

** Product availability subject to meeting country specific registration and language requirements.*

P089. Urine monitoring following heavy intake of cannabis in a pregnant woman – new intake or not?

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Introduction: It may be of crucial importance to clinicians and law enforcers to know whether or not there has been a new intake of an illicit drug between two positive urine specimens from one individual. However, differentiating new drug use from residual drug excretion may be difficult, especially for drugs with long elimination half-lives, such as cannabis. In theory, following cannabis smoking, the THCCOOH/creatinine concentration ratio (CC ratio) in urine should gradually decrease until a new drug intake occurs. However, fluctuations may occur. Some approaches for interpreting CC ratio changes over time have been suggested, but there is no validated approach to distinguish new intake from residual excretion in heavy cannabis users. Moreover, the influence of pregnancy is not known.

Aims: We present a case of a pregnant heavy cannabis user, where dire consequences could follow erroneous conclusions regarding new drug exposure.

Methods: A 20-year-old Norwegian female tested positive for cannabis in week 31 of pregnancy. She admitted having a history of heavy cannabis use, with a daily consumption of approximately five grams hashish. She claimed to have stopped using cannabis when her drug use was discovered, but was subjected to serial urinary testing, as Norwegian law states that a pregnant woman using illicit drugs can be institutionalised against her will. Thus, differentiating new drug use from residual drug excretion in this case was crucial. A liquid chromatography-mass spectrometry method was used to determine THCCOOH concentrations in her urine, with 2-7 days intervals.

Results: In the first urine specimen the THCCOOH concentration was 348 ng/ml, and the CC ratio was 110 ng/mg. In later samples, the CC ratios sometimes superseded that of the prior specimen, but overall there was a slow decrease in the CC ratio. The woman was not institutionalised. The last positive sample was taken twelve weeks (84 days) after the first sample, two weeks after delivery. The THCCOOH concentration in this sample was 3.9 ng/ml, and the CC ratio was 5 ng/mg.

Conclusions: This case illustrates an extreme situation of heavy cannabis use during pregnancy. We believe that the THCCOOH excretion curve may represent some kind of limit for how prolonged its excretion may be in a chronic cannabis user during pregnancy. Although we can not define an exact algorithm for interpreting these samples, we would advise following up such cases with frequent urine sampling and monitoring of the declining slope of the CC ratio until drug-free conditions appear.

Keywords: cannabis, marijuana smoking, substance abuse detection, urinalysis

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P090. Biochip array-based immunoassays for multiplex detection of drugs of abuse in oral fluid with Evidence biochip array technology

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Introduction: The use of oral fluid testing for drugs of abuse is becoming increasingly common in the workplace, prisons and in drug rehabilitation clinics. Ease of discrete sample collection coupled with the elimination of specialist training and same-gender collections make this matrix type, in these situations, a more favourable option over alternative matrices.

Evidence biochip array technology enables simultaneous detection of multiple drugs in a single sample using reagents optimised specifically for each individual sample type. It is based on miniaturised pre-defined simultaneous immuno-reactions on a biochip (9 mm × 9 mm), which reduces sample and reagent consumption, increases test output and reduces costs.

Aims: This study reports the analytical performance of two biochip arrays dedicated to oral fluids for the simultaneous detection of drugs of abuse. One biochip array enables multiplex detection of amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates and phencyclidine. With the other biochip array buprenorphine, ketamine,

lysergic acid diethylamide (LSD), 3,4 methylendioxymethamphetamine (MDMA), methaqualone, fentanyl, oxycodone, generic opioids and propoxyphene are simultaneously detected.

Methods: The biochip is used as solid support and vessel where the immuno-reactions take place. Competitive simultaneous immunoassays are used for drug testing with incubation time of 30 min. The capture antibodies are immobilised and stabilised in precise locations, defining arrays and the assay reagents are added to perform the immuno-analytical tests. The drug in the sample and drug-HRP conjugate compete for the binding sites of the capture antibodies. The signal generated in each array is inversely proportional to the analyte concentration and is detected using digital imaging. The semi-automated bench top analyser Evidence Investigator was used. The instrument incorporates dedicated software to process, report and archive multiple data for retrospective access. Cut-off levels can be selected avoiding recalibration and the use of different calibrators. The simultaneous immunoassays are qualitative and the values are normalised and calculated as percentage of the signal from the cut-off test region relative to the signal intensity emitted from the sample test region.

Results: With the biochip array for simultaneous detection of amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates and phencyclidine the simultaneous immunoassays were specific for the target analytes. Two distinct test sites for benzodiazepines allow discrimination of oxazepam and lorazepam with different cross-reactivities for other benzodiazepines. The agreement with alternative immunoassay methodology was ≥85%.

Initial evaluation of the analytical performance of the second biochip array for simultaneous detection of buprenorphine, ketamine, LSD, MDMA, methaqualone, fentanyl, oxycodone, generic opioids and propoxyphene showed specific recognition of the targets. Separate test sites for oxycodone compounds on the biochip showed 100% cross-reactivity with oxycodone. Thebaine and morphine presented% cross-reactivity of 62.6 and 48.3 respectively in the generic opiods test site.

Intra-assay and inter-assay precision for both biochip arrays, expressed as %CV was typically ≤15%. For both arrays, the system allowed the choice of cut-off within the assay measuring range for simultaneous qualitative screening.

Conclusions: Data show applicability of Evidence biochip array technology to the multiplex detection of drugs of abuse per sample of oral fluid using a single device. On one biochip array two distinct test sites for benzodiazepines allow discrimination of oxazepam and lorazepam with different cross-reactivities for other benzodiazepines. On the other biochip array, separate test sites for oxycodone compounds could increase specificity for possible drug identification. Biochip array technology represents an excellent multi-analytical tool for screening batches of samples, 54 samples can be handled at a time with Evidence Investigator system. The miniaturization of the immunoassays using this multi-analyte approach reduces the volume of the sample and reagent volume per test.

Keywords: drugs of abuse, biochip, multiplex, oral fluid

P091. Blood as sample matrix for simultaneous detection of drugs of abuse with Evidence biochip arrays

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Introduction: The use of different sample types for drugs of abuse testing is now commonplace. Whole blood is more often used in forensic toxicology. There are limitations with generic methodologies for clinical and forensic analysis of different matrix types-typically using samples, calibrator and control materials diluted to different concentrations. This may result in a sub-optimal analytical reagent mixture for a particular matrix type. Biochip array technology enables simultaneous detection of multiple drugs of abuse in a single sample using reagents optimised specifically for each individual sample type. It is based on miniaturised pre-defined simultaneous immuno-reactions, which reduces sample and reagent consumption, increases test output and reduces costs.

Aims: This study reports performance of this technology dedicated to blood samples. for the simultaneous detection of buprenorphine, ketamine, lysergic acid diethylamide (LSD), 3,4 methylendioxymethamphetamine (MDMA), methaqualone, fentanyl, oxycodone, generic opioids and propoxyphene.

Methods: Competitive simultaneous immunoassays are employed with incubation time of 30 min. The capture antibodies are immobilised and stabilised on the surface of the biochip in a defined array. The assay reagents are added to the biochip to perform the tests. Chemiluminescent detection is used with a CCD camera incorporated into the dedicated fully automated analyser Evidence. The instrument incorporates dedicated software to process, report and archive multiple data for retrospective access. Cut-off levels can be selected avoiding re-calibration and the use of different calibrators. The simultaneous immunoassays are qualitative and the values are normalised and calculated as percentage of the signal from the cut-off test region relative to the signal intensity emitted from the sample test region.

Results: The simultaneous immunoassays were carried out using a sample volume of 60 µl of prediluted sample. Calibration ranges spanned from 0-2000 pg/ml (LSD) to 0-1000 ng/ml (MDMA). The immunoassays were specific for the target analytes. The biochip contained three distinct test sites for opioid compounds, oxycodone and generic opioids which detected oxycodone (%cross-reactivity 100) and also dihydrocodeine, hydromorphone, codeine, ethylmorphine, hydrocodone as main analytes (%cross-reactivities 82 - 2282). The intra-assay and inter-assay precision expressed as%C.V. were typically ≤15 for three different levels. The system allowed the choice of cut-off within the assay measuring range for simultaneous qualitative screening.

Conclusion: Results show applicability of biochip array technology as a useful multi-analytical tool for drug testing in a single blood sample. Multiple data are generated using a reduced volume of blood sample and reagents dedicated to this matrix type.

Keywords: drugs of abuse, blood, biochip array, multiplex

P092. Toxicology in emergency room in the Valais Hospital

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Introduction: The 1st of January 2004, the Valais Hospital was created for the implementation of cantonal hospital planning in Valais. The Valais is a montain canton in the south of Switzerland, very known for its wine production and tourism. People in Valais (300 000 inhabitants) speaks an old german in the part called Haut Valais and french in the part called Bas Valais.

Aims: This study presents drug-related visits to Valais Hospital emergency departments (EDs) for 2007 and 2008, based on data from the Central institut laboratory and from the Valais Health Observatory (OVS).

Methods: All the patients entered into the Emergency Room (ER) during 2007 and 2008, in the Valais Hospital's sites of Brig, Visp, Sierre, Sion and Martigny were considered. The toxicological answer is constituded of two kinds of analyses : screenings of drugs of abuse (DOA : acetominophen, amphetamine, metamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, PCP, TCA) by immunological test (Triage®, Biosite) in urine, and measure of ethanol in serum with colorimetric method (Cobas 6000 Roche®). The result for DOA test were qualitativ (positiv or negativ) and for alcohol the result was a quantitative result (g/kg).

Results : In 2007, Valais Hospital has delivered a total of 64 720 emergency departments (ED) visits. We observed that 1922 analysis were associated with

drug misuse or abuse: 276 demands for DOA and 1646 demands for alcohol. Among these demands 1138 cases were positiv for alcohol and 131 cases were positiv for DOA. The mean blood alcohol concentration is 2.07 g/kg (0.15 – 5.15 g/kg). The mean blood alcohol concentration for people under the age of 18 is 1,75 g/kg (0.27 – 2.80 g/kg; n = 51) and for people under the age of 21 was 1,84 g/kg (0.21 – 4.20 g/kg; n = 149). In 2008, Valais Hospital has delivered a total of 66370 emergency departments (ED) visits. We observed that 2059 analysis have been associated with drug misuse or abuse : 353 demands for DOA and 1706 demands for alcohol. Among theses demands 1201 cases were positiv for alcohol and 145 cases were positiv for DOA. The mean blood alcohol concentration is 2.05 g/kg (0.14 – 5.47 g/kg). The mean blood alcohol concentration for people under the age of 18 was 1,66 g/kg (0.17 – 3.42 g/kg; n = 70) and for people under the age of 21 was 1,83 g/kg (0.17 – 5.47 g/kg; n = 171).

Conclusions: In Valais Hospital, less than 3% of emergency visits has been associated with drugs misuse or abuse. The mean blood alcohol concentration has stood stable in the two year study. When emergency visits has been associated with drugs, alcohol has been the most frequently drug investigated and found. In 2007 people under the age of 21 represented 13,1% of positiv results for alcohol, and 14.2% in 2008.

Keywords: DOA, blood alcohol, under age drinking, emergency departments

P093. Determination of GHB and related molecules in dried blood spots

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Introduction: Gamma-hydroxybutyrate (GHB) is a short chain fatty acid endogenously present in humans which is both a precursor and a degradation product of the inhibitory neurotransmitter gamma-amino butyric acid (GABA). Although it is used clinically for the treatment of narcolepsy, associated with cataplexy (Xyrem®), illegally produced GHB is especially notorious for its misuse amongst bodybuilders and *clubbers*, and for its use as a *date-rape* drug. Once ingested, GHB is rapidly eliminated, with reported plasma half-lives ranging from 20 min to 1 h. Given its rapid metabolisation, its endogenous presence and the possibility of *ex vivo* formation, proving GHB misuse is often an analytical challenge. Similar to what is done in newborn screening programs, the use of dried blood spots may represent a new, minimally invasive way of sampling and storing blood from abusers or victims (*e.g.* applicable in sexual aggression sets of the police). We therefore developed a simple one-step gas chromatographic procedure for determination of GHB and related molecules in dried blood spots.

Aim: The purpose of our work was to develop a rapid and simple procedure for the simultaneous determination of GHB and related endogenous and exogenous molecules in dried blood spots, using gas chromatography, coupled to mass spectrometry (GC-MS).

Methods: A dried blood spot (obtained by applying 25 µl of blood to Whatman paper and drying) was excised, transferred to a vial and directly derivatized using a mixture of trifluoroacetic acid and heptafluorobutanol (2:1), for 30 minutes at 85 °C. After drying under a gentle stream of nitrogen, the sample was redissolved, followed by centrifugation and transfer of the resulting supernatant to a vial, from which 1 µl was injected onto a HP-5ms column, followed by MS detection using electron impact. Optimization of GC parameters included optimization of injection solvent, injection temperature, purge activation time, pulse time, inlet pressure, flow rate and temperature program. A selected ion monitoring (SIM) method was developed by selecting two qualifier ions and one quantifier ion, based upon the fragmentation pattern obtained after full scan monitoring of injected standard mixes.

Results: Optimization of GC parameters led to the choice of ethyl acetate as injection solvent, injection temperature of 250 °C, purge activation time of 1.5 minutes, pulse time of 2 minutes, inlet pressure of 25 psi, flow rate of helium of 1.3 ml/minute and a temperature program starting at 65 °C for 1.5 minute, ramping to 95 °C at 5 °C/minute and subsequently at 30 °C/minute to 300 °C, which was maintained for 2 minutes, resulting in a total run time of 16.3 minutes. We already successfully determined GHB and BHB, spiked at concentrations of 5 µg/ml, a commonly applied cut-off level for distinguishing endogenous from exogenous GHB in blood.

Conclusion: We developed and optimized a GC-EI-MS method for the simultaneous determination of GHB and related endogenous and exogenous molecules in dried blood spots using a simple one-step procedure. We are currently in the process of validating this method and will apply it onto real patient samples in near future.

Keywords: GHB, GC-EI-MS

P094. Two cases positive to ketamine; possibilities of detection and identification in human serum and urine

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Introduction: Ketamine is a short-acting, dissociative, anesthetic used primarily for veterinary anesthesiology. In humans, ketamine is abused recreationally for hallucination and misused as a "date-rape" drug. The drug (powder or liquid) can be insufflated, injected, or placed in beverages. It is also possible to smoke with marijuana and tobacco. Ketamine is metabolized by *N*-demethylation to norketamine and further dehydrogenated to dehydronorketamine. Within a short time, we analyzed two clinical cases where ketamine was identified. These are the first experiences with the occurrence of ketamine in our laboratory.

Aim: The purpose of the work was to develop a rapid and reliable screening method to detect ketamine together with other toxicologically relevant substances and to show the power of ion trap mass spectrometric techniques (coupled to GC and LC systems) in structural analysis and identification of analytes.

Methods: Samples (serum, urine) were extracted by a simple liquid-liquid extraction with ethylacetate-methylenechloride (80:20). The dried extracts were reconstituted either in 50 µl of ethylacetate for GC/MS or 150 µl of methanol and 400 µl of water for LC/MS^n. Mass spectrometric detection of the analytes was performed in full MS^n mode either on GC/EI-MS or LC/LIT-MS^n after positive electrospray ionization. The MS^n transitions for ketamine were 238.20 to 220.08 (MS^2) to 207.13 (MS^3).

Results: Extracted human samples were assayed using GC/MS 3D IT (Finnigan Magnum), GC/MS ITQ and LC/MS system LTQ XL (linear ion trap).

Case 1: 17 years old girl, found in coma. Findings: serum ethylalcohol more than 3 g/kg, immunoassay screening negative; ketamine identified in urine by HPLC and confirmed using GC/MS. Case 2: 25 years old man, treated for epilepsy, admitted to hospital for accident at work. Findings: in serum ethylalcohol negative, benzodiazepines positive, in urine benzodiazepines and cannabinoids positive; diazepam, nordiazepam, carbamazepine, tiapride and ketamine were found in serum using LC/MS^n screening.

Conclusion: A simple and rapid sample preparation available for both GC/MS and LC/MS screening methods was developed to detect trace levels of ketamine and its metabolites in human serum and urine. The figures will show the power of ion trap mass spectrometry (multiple fragmentations) in identification of the analyte's structure. Because of appearance of new drugs of abuse on the scene there is the need to have a simple and efficient procedure to identify them.

Keywords: ketamin, GC/MS, LC/MS^n, urine, serum, drug screening

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P095. LC-MS/MS screening method for new designer drugs in serum

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Introduction and aims

According to the World Drug Report 2008 of the United Nations the abuse of "other drugs", including substances such as methaqualone, khat, LSD, ketamine and various new designer drugs, account for three per cent of all drug seizure cases. Though the abuse of new designer drugs has stabilized over the last years and remains on a very low level an analytical method is required in order to detect single cases. The LC-MS/MS screening method presented covers 31 new designer drugs that were available as reference standards. All substances are modified molecular structures of amphetamine, tryptamine and piperazine. As phencyclidine and ketamine also have hallocinogenic effects and could be interesting for designer drug consumers they were included to complete the screening spectrum. Pyrrolidinophenone or phenylcyclohexyl derivates could not be purchased yet. Among the amphetamine derivatives are 3,4-DMA, 2,5-DMA, DOB, DOET, DOM, Ethylamphetamine, MDDMA, 4-MTA, PMA, PMMA, 3,4,5-TMA, TMA-6 and members of the 2C group: 2C-B, 2C-D, 2C-H, 2C-I, 2C-P, 2C-T-2, 2C-T-4 and 2C-T-7. AMT, DPT, DiPT, 4HO-DiPT, MiPT, 4HO-MiPT, DMT and 5MeO-DMT belong to the tryptamine group, TFMPP, mCPP and MeOPP to the piperazine group.

Method: Using a Sciex LC-MS/MS API 365 we are able to identify all 33 substances in serum. The method involves addition of various deuterated internal standards and solid phase extraction. The samples are loaded on Waters Oasis MCX cartridges which were previously conditioned with MeOH and adjusted with phosphate buffer at pH 6. After three washing steps (H_20, acetic acid, MeOH) the analytes are eluted with 1,5 ml of dichlormethane/isopropanol/ammonia (80:20:2). The eluate is evaporated to dryness, the residue redissolved in mobile phase and injected in the LC-MS/MS system (Polar RP 150 mm × 2 mm, 4 µm, Phenomenex). The analytes are separated by gradient elution, using 1 mM ammonium formate/0.1% formic acid and methanol/0.1% formic acid as mobile phases A and B (flow 0.25 ml/min). Isopropanol is added post-column with a constant flow rate of 0.2 ml/min. Data acquisition is performed in MRM mode with positive ionisation: One transition is monitored for the internal standards, two for the majority of analytes and three for all substances that have identical precursor masses.

Results and Conclusion: The method was tested for specifity, matrix effects, recovery and extraction yields. Analysing signal-to-noise ratios yielded the following limits of detection: 28 of 35 substances are detectable at a serum concentration of 5 ng/ml, six substances (4HO-DiPT, 4HO-MiPT, DOET, DPT, Ethylamphetamine and PCP) can be identified at 10 ng/ml (S/N≥3). Initially it was planned to include benzylpiperazine and MDBP as well as cathinone and methcathinone, but the results were unreproducible (for the piperazine derivates) or insufficient (cathinone and methcathinone could not be identified below 50 ng/ml).

Keywords: designer drugs, LC-MS/MS, screening method, serum

P096. Confirmation analysis of benzodiazepines in urine with ultra performance liquid chromatography tandem mass spectrometry

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Introduction: Benzodiazepines are among the most frequently prescribed drugs in the world for the therapy of anxiety and sleeping disorders due to their sedative, hypnotic and anticonvulsant properties. Benzodiazepines are, however, also associated with misuse. The Norwegian Institute of Public Health receives about 25 000 urine samples each year from prison and probation services, social services and workplace testing programs. After immunoassay analysis (CEDIA) the samples screening positive for benzodiazepines are confirmed using LC-MS/MS.

Aim: The purpose of our work was to transfer a former HPLC-MS/MS method [1] to ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) in order to save analysis time and to include with satisfactory separation three benzodiazepines and one metabolite not previously confirmed by the method. A transfer from acetonitrile to methanol as organic solvent in the mobile phase was also performed.

Methods: The method includes hydrolysis of urine samples (0.5 mL) with ß-glucuronidase at 60 °C for 2 hours before solid-phase extraction (SPE) with a polymer-based mixed-mode column (Oasis MCX, Waters). Deuterated analogues were used as internal standards for all analytes when available; otherwise one of the deuterated internal standards from another benzodiazepine was chosen. The analytes were quantified in multiple reaction monitoring mode. Two transitions were recorded for the analytes and one for the internal standards. Chromatographic separation was achieved using an Aquity UPLC HSS T3 column (2.1 × 100, 1.8 µm). The mobile phase consisted of ammonium formate pH=3.1 and methanol. Mass detection was performed by positive ion mode electrospray tandem mass spectrometry and included the following benzodiazepines/metabolites: 7-aminoclonazepam, 7-aminoflunitraze-pam, 7-aminonitrazepam, alprazolam, 1-hydroxyalprazolam, 1-hydroxymidazolam, 3-hydroxydiazepam, bromazepam, diazepam, fenazepam, flunitrazepam, lorazepam, N-des-methyldiazepam, nitrazepam and oxazepam. Factorial design was utilized to optimize the separation and to check robustness.

Conclusion: A fast and specific method for confirmation of benzodiazepines in urine samples has been developed and validated.

Reference: [1] Determination of benzodiazepines in human urine using solid-phase extraction and high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. S. Hegstad, E.L. Øiestad, U. Johansen and A. S. Christophersen. Journal of Analytical Toxicology, Vol. 30, 2006, 31–37.

Keywords: UPLC-MS/MS, benzodiazepines, urine, SPE

P097. Comparative evaluation of target/non-target screen and quantitation techniques for 150 toxicological drugs of abuse

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Introduction: European and world environmental watchdog communities are looking increasingly to the fast screening of toxicological drugs of abuse and their metabolites routinely. The common issue is whether to utilize LC/MS for targeted or non-targeted screening of samples from a variety of complex biological matrices. LC/MS techniques offer the best solution for many matrices and their potential analytes, and this work compares an accurate mass unknown screening technique to a target analysis approach for a suite of 150 analytes. Here, we present two instrumental approaches that address analyte screening and compare/contrast each approach in one compact high-resolution HPLC method. Background levels of some analytes remain significant even after filtration, so it is difficult to obtain reliable low-level (fg) reporting of positives. Here we also discuss increasing reliability of screening with a modified instrument configuration.

Methods: A robust high resolution liquid chromatographic method was developed and used during a comparative proof of principle evaluation between targeted (QQQ) and non-target (QTOF) LC/MS techniques. Samples to illustrate a screen for a suite of 150 targets were employed for each instrument. Appropriate MRM transitions (QQQ) were identified using automatic instrument optimizations and these were applied to the chromatographic method dynamically to maximize analyte signal quality at lower concentrations. QTOF acquisition speed was adjusted to yield similar data quality for direct comparative reasoning. Analysis turn-around

time was below 10 min and background interference was eliminated using a flow trapping setup on the HPLC. Spiked extracted samples at various concentrations were analysed using a 6460 triple-quadrupole & 6530 QTOF mass spectrometers connected to a 1200 Series SL HLPC via a thermally focused ESI interface under identical conditions.

Preliminary results: We report the target suite of compounds yielded a screen coverage (QQQ) at 100 fg on-column levels of above 85% using MRM transitions implemented into the method dynamically. Equally, a non-target screen (QTOF) yielded a 90% coverage for the same sample set, but at a higher on-column amount of 5 pg. Parameter setup for data mining algorithms and compound identification in non-target accurate mass screen show significant improvements in coverage of the 150 toxicology suite (>20%) together with combination searches inclusive of multi ion species and adducts. An evaluation of residual chromatography background interferences for each component shows zero presence when implementing the flow trap approach thus reducing identification of false positives using both screening techniques. MRM transitions were and standard curves generated from serial dilutions of the toxicology suite exhibit R^2 values above 0.997 for both QQQ and QTOF instruments.

Keywords: 150 drugs of abuse, mass spectrometry, triple quadrupole, quadrupole time of flight, blood, urine oral fluids

P098. Physico-chemical analysis of illicit heroin samples seized in Egypt

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Introduction: Analysis of illicit heroin seizures will provide sufficient information to indicate common origins of different samples. In addition, the determination of various diluents and adulterants may help in tracing the route of the sample followed on its way to the users through successive dealers and the identification of the active constituents is thus necessary for proper medical treatments.

Aim: The purpose of our work was to determine the impurity profiling of illicit heroin seizures that essential for both tactical and strategic applications.

Methods: Twenty three seizures were collected from East Delta and North Sinai regions and then analysed by varying techniques including; thin layer chromatography (TLC) (n-hexane – methylene chloride – methanol – ammonia) (8: 7: 1: 0.1), reversed phase high performance liquid chromatography (HPLC) using ODS-silica column (C_{18}) under ultraviolet detection at wavelength 228 nm with gradient chromatographic method using methanol - phosphate buffer (pH 2.93) and gas chromatography-mass spectroscopy (GC-MS).

Results and discussion: The analysis by TLC, HPLC and GC-MS revealed that the samples consisted of opiates (heroin, 6-monoacetylmorphine, and acetylcodeine), opium alkaloids (papaverine, noscapine) adulterants (paracetamol and caffeine) and diluents (metronidazol and griseofulvin). All samples contained very low concentrations of heroin (ranged between 0.25 to 4.4%). The TLC system, reversed phase HPLC and GC-MS conditions that discussed in this work are the optimum conditions used to resolve the major and minor components in illicit heroin samples. The origin of illicit heroin samples was predicted by correlating the UV peak area ratios of heroin to acetylcodeine in each sample with those of samples of known origin. The probable countries of origin are China and Hong Kong. The countries of origin of heroin seizures in both Egypt and Israel are the same.

Conclusion: Analytical data and information on illicit heroin samples can set the scenario for determining the origin of illicit heroin samples (strategic and tactical intelligence purposes).

Keywords: physico-chemical analysis, illicit heroin, HPLC, GC-MS, country of origin, Egypt

P099. Prediction of metabolite mass spectra (MS) of papaverine for library matching using the MS^n capabilities of ion-trap LC-MS

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Introduction: Papaverine is an alkaloid present in the opium poppy, *Papaver somniferum*, that survives the illicit manufacture of heroin from opium. Papaverine is *O*-demethylated to hydroxypapaverine and to dihydroxypapaverine, and subsequently glucuronidated, and these metabolites are excreted in the urine of illicit heroin users. Reference materials and mass spectra are not routinely available for these metabolites. Ion trap mass spectrometers can selectively isolate and fragment ions in MS^n collision events to produce reference spectra. Adjusting the collision energy and the product ion selected at each stage can reproduce the metabolism of the papaverine stage by stage to enable spectra to be acquired.

Aim: The aim of this work was to produce library spectra of hydroxypapaverine, dihydroxypapaverine and their glucuronides to identify use of illicit heroin in clients prescribed pharmaceutical diamorphine.

Methods: A 100 mg/L solution of papaverine (Sigma-Aldrich, UK) was infused into an LCQ fleet ion-trap mass spectrometer (Thermo Scientific, UK) using the integral syringe driver (5 µL/min) with an electrospray ionisation source. The collision energy to fragment papaverine ($[M+H]^+$ *m/z* = 340) was adjusted to give optimal recovery of the $[M-CH_2]^+$ ion (*m/z* 326). This ion was further fragmented, with the second stage collision energy optimised to minimise the $[M-CH_2]^+$ ion and produce a characteristic MS(3) spectrum. The $[M-CH_2]^+$ ion was separately fragmented, optimising the collision energy for isolation of the $[(M-CH_2)-CH_2]^+$ ion (*m/z* 312), corresponding to the dihydroxypapaverine metabolite. This ion was isolated and a third collision event optimised to produce an MS(4) spectrum. The acquired spectra were saved to in-house MS(2) and MS(3) mass spectral libraries. These library spectra were validated by analysing hydrolysed samples (1000 units β-glucuronidase (*Helix aspersa*) in 0.75 mL 1 mol/L pH 5.0 acetate buffer) previously shown to be positive for papaverine metabolites by GC-MS and confirming a good library match. The retention times of these metabolites were recorded by LC-MS analysis (X-LC, Jasco, UK) with a multi-step gradient of 1% (v/v) formic acid / water with 10 mmol/L ammonium formate and 1% (v/v) formic acid / acetonitrile on a 2.1 × 50 mm 5 µm Hypersil PFP Gold column (Thermo Scientific). The same samples were analysed without hydrolysis by monitoring the glucuronide metabolite parent ions (*m/z* 502 and 488 for hydroxypapaverine and dihydroxypapaverine, respectively) using MS(3). Matching these spectra to the library allowed capture of the MS(2) spectra and retention times of the glucuronides.

Results: Reference spectra were produced for hydroxypapaverine, dihydroxypapaverine and their glucuronides, and used in an automated urine drug screening method. These metabolites were detected in urine after use of illicit heroin, but not after pharmaceutical diamorphine medication.

Conclusion: The MS^n capability of an ion trap has enabled the mass spectra of papaverine metabolites to be recorded. These spectra were used to identify illicit heroin use in patients prescribed diamorphine.

Keywords: papaverine, ion trap mass spectrometer, illicit heroin

P100. Laboratory examination for narcotic drugs and psychotropic substances used by drug users in Pattaya, Thailand, 2004-2008

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Introduction: Pattaya is a famous tourist city of Thailand and the world. There are about 4-5 million visitors per year of which two-thirds are foreigners. The growth of tourism, big business and the large number of people who work

in Pattaya brought about many potential consequences. They are pollution, economic, social especially drugs problem. Regional Medical Sciences Center Chonburi (RMSCB), Department of Medical Sciences, Ministry of Public Health is the main agency which carries out drug analyses in urine samples. In 2004-2008, laboratory of RMSCB examined urine samples of drug users for drugs and psychotropic substances.

Aim: The purpose of work was to conduct surveillance of drugs and psychotropic substances used, and to determine the extent of drug problem in this part of Thailand.

Methods: In 2004-2008, urine samples of drug users sent from Royal Thai Police in Pattaya were examined for drugs and psychotropic substances in laboratory of RMSc CB by color test, immunoassay, TLC, TOXI-LAB® and GC-MS techniques. The kinds of drugs and psychotropic substances were amphetamine, methamphetamine, MDMA, MDA, MDE, cocaine, cannabis, morphine, codeine, ketamine, ephedrine, pseudoephedrine and benzodiazepines.

Results and Discussion: Over 2004 to 2008 the number of drug users in Pattaya increased each year. There are 1314, 1303, 1468, 2205 and 2788 cases, respectively. Drugs users were mainly male for each of the five years. The most common of age range of drug users was 15-34 years. However, there was a tendency to increase the proportion of users between 15-19 years. The most common drugs identified were methamphetamine (> 85%) over all the 5 years. Cannabis use increased while ecstasy, opiates, ketamine, ephedrine and pseudoephedrine decreased. Cocaine used was found only in 2006 (2 cases) and 2007 (1 case). Methamphetamine use was mostly single and more than those cases associated with other drugs, while cannabis use was mainly associated with other drugs. About 97% of cannabis use was associated with methamphetamine.

Keywords: drugs of abuse, urinalysis, Pattaya

Doping analysis

P101. Blood doping with hemoglobin-based oxygen carriers (HBOC): analysis by CE-UV and CE-ESI-TOF/MS

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Introduction: Blood doping is defined by WADA (World Anti-Doping Agency) as the use of products that enhance the uptake, transport, or delivery of oxygen to the blood. Most commonly, this involves erythropoietin (EPO) uptake or blood transfusion. Another approach uses artificial oxygen carriers, known as hemoglobin-based oxygen carriers (HBOC). HBOC were initially developed as artificial blood replacement products to substitute the oxygen-carrying functions of erythrocytes. These products are made of bovine or human hemoglobin (Hb) which is intra- or intermolecularly cross-linked, polymerized, or conjugated. Although several HBOC are under development or in clinical trials, only a few have been approved by authorities. Among them, Oxyglobin® (Biopure, Hb of bovine origin) was the only one approved for veterinary purpose by the FDA, while Hemopure® (Biopure, Hb of bovine origin) was approved for human use in South Africa.

Aim: A complete analytical strategy based on capillary electrophoresis (CE) was developed to detect intact Oxyglobin® in plasma samples collected for doping control. The main issue consisted of ensuring sufficient electrophoretic resolution between Oxyglobin® and Hb that could be released from mechanical haemolysis. A particular attention was paid to choose adequate sample preparation procedure.

Methods: A sample preparation based on immunodepletion was mandatory to remove most abundant proteins that interfered with CE separation and altered electrospray ionization (ESI). On-capillary detection was performed with UV at 415 nm, offering sufficient selectivity for hemoproteins (such as Hb and Oxyglobin®). Online MS detection with TOF analyzer was also used to provide accurate mass on CE peaks and unambiguous determination of Oxyglobin® uptake.

Results: Best CE selectivity was obtained at basic pH without any coating procedure or particular washing. An interesting selectivity was obtained with ESI-TOF/MS since bovine Hb presented distinct chain masses compared to human Hb. Oxyglobin® being polymerized bovine Hb, differentiation from human Hb was possible. Sample preparation appeared as the key point of the method development. Plasma interfering proteins had to be removed to (i) reduce adsorption on the capillary wall, (ii) avoid Oxyglobin® peak degradation, and (iii) counteract ESI suppression. Best results were obtained with immunodepletion. The developped methodology allowed reaching LOD of 0.3 $g \cdot dL^{-1}$ in plasma.

Conclusion: Three levels of selectivity were obtained for Oxyglobin® analysis in plasma by CE-UV and CE-ESI-TOF/MS: electrophoretic mobilities, UV detection at 415 nm, and ESI-TOF/MS detection. The methodology was successfully applied to Hemopure® in real plasma samples.

Keywords: capillary electrophoresis, HBOC, blood doping, time of flight mass spectrometry

P102. GC×GC-TOFMS improves the separation and identification of anabolic agents in doping control

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Introduction: The very low concentrations of the anabolic agents (AA), and the complex matrices in which they are found, require powerful techniques for separation and unambiguous identification. At present gas chromatography coupled to a quadrupole mass analyzer (GC-qMS) is a technique of choice which exhibits high specificity and sensitivity, especially when selected ion monitoring (SIM) mode is applied. The separation is based on using traditional narrow bore capillary columns, with detection and identification relying on monitoring pre-defined diagnostic ions in pre-defined time windows. Most – if not all – of the current methods in doping analysis are "transparent" to potentially new "designer" anabolic agents at the required lowest level of detection. Full scan MS techniques coupled to the classical one dimensional GC (1D GC) are not sensitive enough to detect the low level concentrations (down to 1 ng mL^{-1}) as defined by World Anti Doping Agency (WADA).

Aim: The purpose of this work was to show the improved separation power of comprehensive 2D GC (GC×GC) in doping analysis, to overcome solute "transparency" by acquiring full scan MS by using time-of-flight (TOFMS) detection, and finally to check if the results comply with WADA criteria.

Methods: Urine extracts spiked with anabolic agents were prepared in the concentration range of 2.0 ng mL^{-1} to 20 ng mL^{-1}. The extracts were obtained by solid phase extraction, followed by hydrolysis and liquid-liquid extraction of blank urine samples. Prior to analysis, the residue was derivatized by dissolving in derivatization mixture (MSTFA-NH_4I-ethanethiol) and heating at 80 °C for 30 min.

Results: GC×GC offers much better separation compared to 1D GC, due to separation on two "orthogonal" columns. High acquisition rate (100 Hz) with full mass scanning permits deconvolution of AA spectra interferences, and importantly identification based on MS similarity. Relative ion abundance ratios, retention times, MS identification and other method performance criteria were found to comply with the WADA requirements,

except the minimum of three diagnostic ions with relative abundance higher than 10% (arises due to bias of TOFMS detection against higher masses). Tens of components were identified with average similarity of 920 (on a 0-999 scale), including 10 endogenous sterols, and full mass spectra of 5000+ compounds were recorded. The T/E ratio was obtained from the same run.

Conclusion: An added separation dimension in doping analysis has been implemented in this study. Rather than increasing method performance through detection "transparency" (as in SIM, HRMS, or tandem MS analysis) we have improved method capabilities by enhanced separation power, whilst retaining full mass spectral information. Most of the WADA criteria for doping control analysis are satisfied. The high MS similarity to reference compounds overcomes the limitation of requiring three diagnostic ions for identification.

Keywords: GC×GC, doping analysis, WADA criteria

Alternative matrices in forensic, clinical and food toxicology

P103. Analysis of absinthe by solid phase extraction and LC-M/MS: a simple test for thujone concentration

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Introduction: Absinthe, once a beverage subject to a ban in the United States is now finding a new audience. It is euphemistically called the "Green Fairy" after the color of the liquid. The main constituent of the beverge is Anethole but some samples of the drink may contain α/β thujone. The α/β thujone is thought to give rise to the hallucinogenic properties. Absinthe is also known to contain upto 50 percent acohol by volume. The focus of this presentation is to to demonstrate a simple and efficient procedure for the analysis of absinthe which can determine the presence (or absence) of α/β thujone together with anethole. This procedure should be of great assistance to those actively testing such drinks and offer an alternative method of analysis.

Aims: The focus of this work, is to demonstrate the use of both an alternative solid phase procedure employing a long chain sorbent (C_{30}), and LC-MS/MS in the analysis of an alternative matrix *i.e.* absinthe thus offering analysts a different route for α/β thujone testing.

Methods: Calibrators and controls were used for extracting anthethole and α/β thujone from 50% (v/v) aqueous alcohol (ethanol) samples (0.2 mL). In this method, to samples of calibrators, controls and, genuine samples of absinthe (n=10), was added menthol (1000 µg/ mL) as an internal standard before mixing and dilution with deionized water (5 mL). The samples were applied to conditioned solid phase extraction columns (3 mL containg 25 mg CEC30 solid phase sorbent (UCT Inc.)). The columns were previously conditioned with methanol and deionized water (1 mL, respectively). After washing the sorbent with deionized water (0.4 mL), the samples were eluted with 4 × 50 µL of methanol. The eluates were collected, and transferred directly into autosampler vials for analysis by LC-MS/MS in positive electrospray (MRM) mode. The quantifying transitions were: Menthol: (156.1 → 83.1), α/β thujone: (153.1 → 135.1), and anethole: (149.1 → 121.2), respectively. From the analysis of the calbrators and controls: r^2 value> 0.995, recoveries > 90%, and a limit of detection of 10 µg/ mL, respectively were acheived for α/β thujone. The method was found to be linear upto 1000 µg/mL. Tandem mass spectrometry was performed on an API 2000 MS/MS unit, the liquid chromatography was performed using: 50 × 2.1 mm (3 µm) phenyl column (Selectra ™) for separation of the analytes. A mobile phase consisting of acetonitrile (containing 0.1% (v/v) formic acid) and deionized water (containing 0.1% (v/v) formic acid) was used in gradient mode in the analysis.

Results: Data is presented in this poster along with LC-MS/MS chromatograms showing those samples of genuine absinthe containing α/β thujone and those containing anethole only. The range of α/β thujone concentrations was found to be from 0 to over 750 µg/ mL (348 to 769 µg/ mL (n=5)) while all the samples analyzed contained anethole at various levels (2480 to 7720 µg/ mL (n=10)) *i.e.* far exceeding that of the α/β thujone.

Conclusions: This simple amd efficient procedure for the analysis of absinthe (especially α/β thujone) is the first method using both solid phase extraction and LC-MS/MS. The use of this procedure should assist those analysts involved testing Absinthe beverages for the presence of thujone. This method should also help analysts testing pre-ban samples of Absinthe for authenticity, as the presence or absence of the α/β thujone should assist in the establishment of the legitimacy of the sample.

Keywords: absinthe, SPE, LCMSMS

P104. A validated method for simultaneous screening and quantification of 29 drugs of abuse in oral fluid by solid phase extraction and UPLC-MS/MS

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Introduction: Oral fluid (OF) is an interesting choice as a sample for drug-of-abuse testing, especially for testing those suspected of driving under the influence of drugs. Substances can be detected in OF for short periods of time, typically 12–24 h after consumption. OF is therefore suitable for detecting recent drug use, *e.g.*, for roadside testing. Because only a limited amount of OF is available for drug analysis, it is crucial to have a sensitive multi-component method for sample analysis. In that connection we have developed a sensitive multi-component method for analysis of OF collected with Statsure sampler device The method includes the following compounds: morphine, 6-acetylmorphine, codeine, amphetamine, methamphetamine, MDA, MDMA, MDEA, methadone, cocaine, benzoylecgonine, zolpidem, tramadol, buprenorphine, diazepam, nordiazepam, nitrazepam, 7-amino-nitrazepam, clonazepam, 7-aminoclonazepam, flunitrazepam, 7-aminoflunitrazepam, bromazepam, oxazepam, chlordiazepoxide, alprazolam, lorazepam, zopiclone, and Δ-9-tetrahydrocannabinol. We will describe the validated ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method using SPE extraction for sensitive OF drug screening and quantification of 29 drugs/illicit compounds.

Aim: The purpose of the work was to develop a sensitive multi-component method for analysis of OF collected with the Statsure sampler device covering the most common drug of abuse (morphine, 6-acetylmorphine, codeine, amphetamine, methamphetamine, MDA, MDMA, MDEA, methadone, cocaine, benzoylecgonine, zolpidem, tramadol, buprenorphine, diazepam, nordiazepam, nitrazepam, 7-amino-nitrazepam, clonazepam, 7-aminoclonazepam, flunitrazepam, 7-aminoflunitrazepam, bromazepam, oxazepam, chlordiazepoxide, alprazolam, lorazepam, zopiclone, and Δ-9-tetrahydrocannabinol).

Method: A Gilson SPE robot equipped with Bond Elut Certify SPE (130 mg, 3 mL) columns was used. After conditioning of the columns 0.200 g OF samples + 200 µl Statsure buffer were diluted with 5 mL of ammonium acetate (pH 4.1)/methanol (v/v, 90:10) buffer and introduced into the SPE. The analytes were eluted with acetonitrile added 0.5% aqueous ammonium. Eluates were evaporated and redissolved in 200 µL of mobile phase (2 mmol/L ammonium acetate buffer, pH 6.2/methanol [v/v, 20:80]). Target drugs were quantified using a Waters ACQUITY UPLC system coupled to a Waters Quattro Premier XE triple quadrupole

(ESI+, MRM mode). The column used was a 100 mm × 2.1 mm, 1.8 µm Acquity UPLC HSS T3 C18. The mobile phase was composed of solvents A (2 mmol/L ammonium acetate, pH 6.2) and B (100% methanol). A gradient program was used.

Results: Extraction recoveries were 36%–114% for all analytes.LoQ was 0.0005 mg/kg. The measurement range was 0.0005 to 0.1 mg/kg. Only minor to moderate matrix effects were observed.

Conclusion: In conclusion, our method is capable of analyzing a wide range of drugs of abuse in OF with high sensitivity. The automated extraction procedure and the relatively short chromatography time of 15 min allow a high throughput of samples.

Keywords: UPLC-MS/MS, solid phase extraction, saliva, psychotropic drugs

P105. Detection of methamphetamine and amphetamine in hair after washing with detox shampoo in Korea

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Introduction: Analysis of methamphetamine (MA) and its metabolite, amphetamine (AP) from hair sample becomes a routine work in the investigation of methamphetamine-abuse related criminals in Korea. Recently, it was reported there was a case of a methamphetamine abuser using a so-called 'detox shampoo', of which commercial name is Get Clean Shampoo in the internet markets in order to disturb hair analysis. In this work, the disturbing effect of the detox shampoo to hair analysis was investigated by comparing the amounts of methamphetamine (MA) and amphetamine (AP) from a detox shampoo treated hair sample with those from a hair sample without detox treatment. Hair samples were obtained from methamphetamine abuser and were analyzed by GC-MS. In consequence, there were a significant decrease in the amounts of both methamphetamine (MA) and amphetamine (AP) in the detox shampoo treatment.

Aims: The disturbing effect of the detox shampoo to hair analysis was investigated by comparing the amounts of methamphetamine (MA) and amphetamine(AP) between a detox shampoo treated hair sample and a hair sample without detox treatment

Methods: The hair sample was washed, cut, measured, extracted and derivatived. An aliquot (1 µL) of the sample solution was injected through the DB-5MS capillary column (30 m × 0.25-mm i.d., 0.25 µm) of a 6890N GC coupled with a agilent technology 5975i MSD. The column temperature was programmed to be held at an initial temperature of 90 °C for 3 min, then increased to 170 °C at a rate of 15 °C/min, held for 3.0 min, increased to 210 °C at a rate of 25 °C/min, held for 1.5 min, then increased to 230 °C at a rate of 20 °C/min, held for 0.5 min, finally increased to 300 °C at 35 °C/min and held for 0.5 min. The inlet and the GC interface temperatures were 260 and 280 °C, respectively. The detector was used in electron impact (EI) mode with selected ion monitoring (SIM) for quantification at 70 eV. The monitoring ions were m/z 154, 118, 160 for MA-TFA and MA-d_{11}-TFA, while m/z 118, 140, 143 for AP-TFA and AP-d_8-TFA.

Results: As for the detox treated sample, the amounts of MA and amphetamine were 17.99 ± 3.42 ng/mg (k=2.1, 95% confidence level) and 0.86 ± 0.42 ng/mg (k=2.1, 95% confidence level), while those from the sample without detox treatment were 23.94 ± 4.57 ng/mg (k=2.1, 95% confidence level) and 1.57 ± 0.42 ng/mg (k=2.1, 95% confidence level).

Conclusions: It is found that there is a significant decrease in the amount of MA(- 24.80%) and AP(-45.10%). From this result, the detox shampoo turns out to have a disturbing effect to hair analysis of methamphetamine (MA) and amphetamine (AP).

Keywords: GC/MS, Methamphetamine, detox, shampoo

P106. Effect of bleaching on ethyl glucuronide in hair: an *in vitro* experiment

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Introduction: Ethyl glucuronide in hair (HEtG) has recently gained great attention, because of the high sensitivity and specificity in the diagnosis of chronic alcohol abuse. Due to its high hydrophily, a strong hair treatment followed by a shampooing may lead to removal/degradation of this molecule from hair matrix.

Aim: To set up an *in vitro* study in order to evaluate the ability of bleaching of modifying HEtG test results.

Methods: Thirty hair samples from teetotalers (n=5), social drinkers (n=4) and heavy drinkers (n=21), after an informed written consent, were collected and divided longitudinally into four aliquots. The first aliquot was kept untreated and was processed following the method routinely used in our lab for the determination of HEtG (double washing with methanol/dichloromethane, overnight incubation in water, and LC-MS/MS analysis). To the other three aliquots an commercially available bleaching solution was applied, according to the manufacturer's instructions. One out of the three aliquots was submitted to the analysis by following the same procedure used for the untreated sample. The other two were submitted to a purification step before LC-MS/MS analysis, by using two different SPE cartridges (aminopropyl and dimethyl-butylamine).

Results: HEtG levels in the untreated samples from social drinkers and heavy drinkers ranged from 7.7 to 149.0 pg/mg. All the samples from teetotalers tested negative. The treated samples processed without any SPE extraction and with aminopropyl cartridges showed a huge ion suppression for both EtG and D_5-EtG (IS) signals. Samples treated with the home bleaching solution and extracted with the dimethyl butylamine cartridge allowed to sensitively reduce ion suppression (less than 35%) and to verify that EtG, after a strong treatment like a bleaching, completely disappears.

Conclusions: This *in vitro* study showed that HEtG disappears from hair matrix after a strong hair treatment. It is not clear whether the mechanism involved is chemical degradation or physical removal from the damaged keratinic matrix. However, owing to the high hydrophilic character of the compound, the second mechanism seems more likely to occur. Finally, bleaching solutions could lead to a heavy ion suppression of this metabolite that may be avoided by using a SPE purification before instrumental analysis.

Keywords: ethyl glucuronide, hair, cosmetic treatment

P107. Automated immunoassay for the detection of barbiturates in oral fluid on Roche instrument platform

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Introduction: A homogeneous immunoassay is being developed for the qualitative and semi-quantitative determination of barbiturates in oral fluids on Roche automated clinical analyzers*. In recent years, interest in oral fluids as an alternate matrix for measuring drugs of abuse has increased. Sample collection is less invasive than with urine, more cost-effective, and adulteration is more difficult. Results are more indicative of recent abuse, and better reflect drug doses at the site of action.

Methods: The assay utilizes KIMS technology (kinetic interaction of microparticles in solution) with ready-to-use liquid reagents. The two-reagent system consists of a first reagent containing a polyclonal antibody

with a broad reactivity profile for barbiturate compounds plus an accelerant, and a second reagent containing a protein-drug conjugate covalently coupled to carboxy-modified polystyrene microparticles. The barbiturates oral fluid assay utilizes a cutoff of 20 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. The test range of the assay is 0 - 160 ng/mL. Multi-calibrators based on a proprietary synthetic matrix are provided by OraSure Technologies, Inc.

Results: When run in a semi-quantitative mode with a 6-point calibration on a Roche/Hitachi 917 analyzer, calibration curves are generated reproducibly with an analytical sensitivity (mean – 3*SD) of *ca.* 10% of the cut-off concentration (1.3-2.5 ng/ml), while maintaining sufficient dynamics over the entire measurement range. Individual oral fluid samples spiked at +/-25% of the cut-off concentration are recovered within a range of 100 +/-10%, *i.e.* ca.14.1-16.2 and 24.1-26.6 ng/ml, respectively, with no crossovers. Within-run precision studies (n = 21) at these levels yield%CV values of 2.5 – 5.5%. Preliminary studies showed a broad cross-reactivity with a variety of barbiturate drugs. Accelerated stability studies (temperature stress model) indicate a real-time stability period in excess of 18 months at 4 °C.

Conclusion: In summary, the test reagents and assay procedure show performance characteristics suitable for testing Barbiturates in oral fluids. *These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: drugs of abuse, oral fluids, barbiturates, immunoassay, automation

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P108. Fatty acid ethyl esters in hair and their role in the diagnosis of heavy alcohol use

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Introduction: Hair analysis is gaining increasing importance as a helpful means in the diagnosis of alcohol heavy use and misuse, following, in particular, the development of analytical procedures able to determine alcohol biotransformation products, such as fatty acid ethyl esters (FAEE) or ethyl glucuronide in hair samples.

Aim: This project intends to study the correlation between ethanol daily intake and FAEE hair concentration in twenty-three patients of the Alcohol Center. The ethanol daily intake was assessed during the interviews aiming at the definition of the diagnosis of alcohol related pathologies and of the health state of the individual. Secondly, cocaine positive hair samples (either positive or negative to cocaethylene) were analysed for FAEE determination with the aim of comparing the two different markers.

Methods: FAEE (ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate) were extracted from the keratinic matrix by overnight incubation in n-hexane/dimethylsulphoxide. The incubation layer of hexane was extracted on aminopropyl-NH2 solid phase extraction (SPE) cartridges and the resulting extract was injected in the gas chromatograph-mass spectrometry (GC-MS) apparatus in selected ion monitoring (SIM) mode. The method was validated (LLOQ: 0.01 ng/mg for each substance, imprecision and accuracy better than 13.6%) and identification of all substances was based on at least three ions per analyte. The analysis of cocaine, cocaethylene, benzoylecgonine, and ecgonine methylester was accomplished by the validated method on routine in the laboratory (HCl overnight incubation, SPE, GC-MS in SIM mode, LLOQ of cocaine and metabolites: 0.02 ng/mg).

Results: Hair samples from patients of the Alcohol Center had FAEE concentration between 0.82 and 3.72 ng/mg (median: 1.58 ng/mg), in relatively good agreement with the general health state and with the assessed ethanol daily intake (min: 15 g/day, max: 540 g/day, median: 120 g/day), with the exception of binge drinkers, who were known to drink alcohol (about 30 g) one-two times but presented relatively high FAEE concentrations (0.82-1.31 ng/mg). Hair samples from cocaine users had cocaethylene between non-detectable level to 12.97 ng/mg (median: 0.08 ng/mg) corresponding to a cocaethylene percentage (calculated considering the sum of cocaine, benzoylecgonine, cocaethylene, and ecgonine methylester equivalents as total cocaine) from 0 to 18.98%. FAEE concentration was found between 0.22 and 6.01 ng/mg (median: 0.82). Using a FAEE cut-off of 0.5 ng/mg as suggested by Pragst and Yegles (Ther Drug Monit 2008; 30: 255–263), of the 13 cocaethylene positive samples (cut-off: 0.2 ng/mg) one only resulted FAEE negative; of the 23 cocaethylene negative samples, 8 tested FAEE negative and 15 tested FAEE positive. The latter probably indicates a trend of use of either ethanol or cocaine although never simultaneously.

Conclusions: FAEE hair analysis seems to be an interesting tool in the diagnosis of alcohol related pathologies when associated with the traditional signs and indicators. Moreover, some remarkable issues, such as FAEE hair concentration in binge drinkers or their incorporation in the keratinic matrix (in comparison with cocaine/cocaethylene) are still to be thoroughly investigated.

Keywords: fatty acid ethyl ester, hair, ethanol daily intake

P109. Validation of a liquid chromatographic-tandem mass spectrometric method (LC-MS/MS) for the quantification of ethyl glucuronide in hair according to the guidelines of forensic toxicology

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Introduction: Ethyl glucuronide (EtG) is a minor metabolite of ethanol, and its presence in urine can be used as a laboratory test to detect recent alcohol intake, even for some time after the ethanol is not longer measurable. Additionally EtG accumulates in hair allowing a large retrospective time window for the detection of previous alcohol consumption. On the strength of past experience, strict abstinence is excluded or improbable at $c_{EtG} > 7$ pg/mg according to recent guidelines in Germany. Analysis has to be performed in a laboratory accreditated according to ISO17025 for forensic purposes using fully validated procedures.

Aim: The intention of our work was to develop a fast sensitive and selective procedure for the quantification of EtG in human hair. The method should be validated for selectivity, linearity, precision, accuracy and recovery according to the guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh). Afterwards the method's applicability should be tested on several samples from alcoholics and moderate social drinkers.

Methods: The hair samples were prepared according to the method of Polettini *et al.* (2006).

Chromatographic separation was performed by high performance liquid chromatography (HPLC). Various HPLC columns (Polar RP (Phenomenex) and Hypercarb (Thermo)) were tested.

Quantitation was achieved by MS/MS detection in the multiple reaction monitoring (MRM) mode, using precursor to product ion transition of m/z 221/75 for EtG and 226/75 for the internal standard d5-EtG. The transitions m/z 221/85 and m/z 221/113 were used as qualifiers.

Results: No significant interference was observed in six blank hair samples from teetotalers.

The 7-point calibration curve with spiked hair samples was linear over the concentration range of 2-1000 pg/mg. The average equation for the linear regression was $y = 0.008 \times + 0.014$ with a correlation factor greater than 0.99. According to the German Industrial norm DIN 32645 the limit of detection (LOD) of 1 pg/mg and a lower limit of quantitation (LLOQ) of 4 pg/mg were calculated. The validation data for QC samples at 4, 40 and 400 pg/mg were: intra-day precision 16.79%, 4.41% and 3.34% relative standard deviation (RSD); inter-day precision: 17.62%, 5.80 and 5.29% RSD; bias (inter-day):

-1,5%, -5.27% and -3.36%. The validated LC-MS/MS method was successfully applied to real cases. As known from urine analysis, interfering peaks appeared also in hair samples of some real cases however. Various HPLC columns were used to compare the chromatographic separation and so avoid false positive results.

Conclusion: A fast sensitive and selective procedure for the quantification of EtG in human hair was developed and fully validated. From the results of the validation parameters we can conclude, that the present method will provide a powerful tool to control alcohol abstinence according to the sophisticated German guidelines. Caution has to be made because an insufficient separation can produce false positive results due to interferences.

Keywords: ethyl glucuronide, alcohol, hair analysis, LC-MS/MS, abstinence, guidelines, GTFCh

P110. Confirmation of delta 9-tetrahydrocannabinol (THC) in saliva by selected reaction monitoring on a GC-triple quadrupole mass spectrometer

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Introduction: Analysis of saliva is becoming an increasingly popular biological fluid as an alternative to blood and urine to analyze for drugs of abuse. As opposed to urine, the collection of saliva can be monitored without embarrassment. Also, it has the advantages over blood in its ability to be collected without pain or the need for extensive training. Since THC is one of the most common illegally used drugs, its analysis in saliva has become very important. This analysis can be extremely challenging due to the low concentrations of THC typically found in saliva, and the low volumes of saliva which are generally available for analysis. Also, like many biological fluids, chemical noise from the matrix can be problematic, especially at these low levels.

Aim: To evaluate the use of a GC-triple quadrupole mass spectrometer for the analysis of THC in a saliva matrix.

Methods: Saliva samples were first extracted and the clean up was done by solid phase extraction. After evaporation of the elution solvent, samples were derivatized using a trimethylsilyl derivative to improve chromatography. The derivatized samples were then introduced into a split/splitless GC inlet, and separated with a 5% phenyl column. The MS was operated in selected reaction monitoring mode measuring one quantitation and one qualifying ion for both THC and its deuterated internal standard.

Results Linearity of spiked, extracted samples was demonstrated between 0.4 ng/mL to 20 ng/mL. Precision was demonstrated to be less than 10% RSD at 0.8 ng/mL and 2.5 ng/mL.

Conclusion: This study demonstrates that using a GC-triple quadruple in selected reaction monitoring mode can provide both the selectivity and sensitivity required for the low level analysis of THC in saliva.

Keywords: saliva, THC, GC/MS

P111. Hair urine analysis for methamphetamine in Thai young adults admitted for Yaba abuse

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Introduction: Methamphetamine (MA) is still a dominant drug in Thailand. The tablet form known as "yaba" consists of MA at about 20 mg%. The detection method is usually by urine analysis. However, this method is limited by a narrow time window for detection. Hair urine analysis is another option for diagnosis of MA use. While it is not yet an established practice in Thailand, this method is needed to assist investigators in identifying MA abusers who may not use yaba regularly.

Aim: The purpose of our study was twofold: first, to establish the validity of a method for MA determination in our laboratory and, second, to determine the presence of MA in the hair of young Thai adults participating in a randomized behavior change intervention trial in northern Thailand.

Methods: Hair was washed 3 times with distilled water and acetone. After being dried and cut into small pieces, 20 mg of hair was subjected to 1 M HCl extraction. The extract was placed into a new vial containing 1 M K_2CO_3. The hair sample was analyzed by head space-solid phase micro-extraction (HS-SPME) coupled with a GC-MS. Polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was used in HS-SPME under conditions of incubation temperature at 90 °C for 5 min, extraction time of 10 min., and desorption temperature of 250 °C for 5 min. Quantitative and qualitative urine analysis by GC-MS was performed using selected ion monitoring (SIM) mode. Hair specimens were collected from 1,111 young Thai adults who had admitted using yaba at least 3 times during the past 3 months. Hair was cut from the posterior vortex and kept in a clean plastic bag with root ends marked. Hair was then cut into 3 cm long pieces for MA analysis. Interviewers also asked the subjects about their history of yaba abuse.

Results: MA concentration of 0.5-10 ng/mg of hair showed good linearity, with a correlation coefficient of 0.9974. Accuracy for MA urine analysis was 98.39-103.75% for intra-day assays and 93.50-98.06% for inter-day assays. Precision of urine analysis was 8.54-12.92% for intra-day assays and 9.07-11.23% for inter-day assays. The limits of detection and quantitation for MA were 0.3 and 0.5 ng/mg of hair, respectively. This validated method was used to analyze hair from 1,111 yaba abusers. Seventy-five percent of the subjects were male. Their ages were from 18 to 25 years in both sexes. The average history of yaba abuse was 4.59±2.35 (mean±SD) years in males and 3.58±2.17 years in females. The median time since last yaba use was 9 days in both groups. MA was detected in 35.5% of these subjects. MA in hair ranged from 0.51-54.61 ng/mg of hair with a mean of 4.90±6.78 (mean±SD) ng/mg. Twenty-nine percent of subjects who had abused less than 10 yaba tablets within the past 3 months showed positive in hair. The percentage of hair samples positive for MA was higher in the group who had used 10-30 tablets (37.5%), 30-90 tablets (36.7%) and more than 90 tablets (*43.6%) of yaba. Yet, the mean MA levels in hair of these different use intensity groups was not significantly different. There was no linear correlation between the amount of yaba used during the past 3 months and the level of MA in hair.

Conclusion: The method of MA urine analysis of hair was validated in our laboratory with good linearity and detection levels. The technique was used for baseline screening of yaba abusers. The overall detection rate was 35.5% and the mean MA level was 4.90±6.78 (±SD). With increased use of yaba tablets (>10), there was a tendency to a higher percentage of MA positive in hair.

Keywords: hair analysis, methamphetamine, Thai, yaba abuse

P112. A targeted screening method for the most common drugs of abuse in hair using LC-MS/MS

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Introduction: The use of LC-MS/MS techniques in forensic toxicology is increasing due to the sensitivity and selectivity of modern instruments. An LC-MS/MS method was developed to screen and semi-quantify the most common drugs of abuse in hair, including amphetamines, opiates, cocaine, benzodiazepines and cannabis.

Method: An aliquot of hair (~100 mg) obtained from volunteers is briefly washed. The hair is allowed to dry, weighed, cut into small fragments and placed into a glass tube with 2 ml of methanol and incubated overnight at 50 °C. The methanol is transferred into another glass tube and evaporated to dryness under nitrogen at 40 °C and reconstituted with 100 µL of mixture of 90% Eluent A [50 mmoL ammonium formate in water (adjusted to pH 3.5)] and 10% Eluent B [containing 0.1% of formic acid in acetonitrile] containing internal standard (MDMA-d5 100 ng/mL). Each sample is transferred to an

autosampler vial and 10 µL injected into a liquid chromatograph coupled with tandem mass spectrometer (ABI 3200 Q-Trap) using electrospray ionization and detection in MRM mode. Post chromatographic analysis is performed on Applied Biosystems Analyst software. For every batch of hair extracts, nine calibration standards with drug concentrations of 0.005, 0.01, 0.02, 0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 ng/mg are prepared from drug free hair. Approximately 100 mg of drug free hair is used for each calibrator and no less than three different blank drug free hair samples are also assayed with every batch.

Results: The average extraction recovery of drugs of abuse in hair was 80% using spiked hair samples. The limit of detection (LOD) using the described system for most analytes is 0.1 ng on column. The limit of quantification (LOQ) for all analytes is 0.02 ng/mg. The method is linear to 100 ng/mL (r^2=0.99).

Conclusions: This semi quantitative targeted screening method for the most common drugs of abuse in hair is a powerful tool for forensic and clinical applications where detection limits are in the picogram range and the determination of a drug exposure is crucial for a timed event.

P113. Detection and validated quantification of 31 drugs of abuse in oral fluid

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Aims: Detection of drugs of abuse in oral fluid is becoming increasingly popular due to the advantages of this "alternative" matrix over "traditional" matrices. Oral fluid can provide a quick and non-invasive specimen for drug testing. In recent years, the use of oral fluid has been accepted for many roadside and workplace drug testing programs. Confirmation tests need to cover a broad range of drugs and have to be able to detect low concentrations, as collection devices additionally dilute the collected oral fluid sample.

The aim of this study was the development and validation of fast and reliable method for detection of the most common drugs of abuse in oral fluid.

Methods: After liquid-liquid extraction (LLE) of 200 µL of neat or diluted oral fluid, 4 amphetamines, 12 benzodiazepines and metabolites, cocaine and 3 metabolites, 7 opioids, and 3 miscellaneous drugs including THC were separated using a Shimadzu Prominence HPLC system with an C18 separation column (Eclipse XBD C18, 4.6 × 150 mm, 5 µm), using gradient elution with a mobile phase of 50 mM ammonium formate buffer pH 3.5 / acetonitrile. The drugs were detected using an Applied Biosystems API 5000 LC-MS-MS system (ESI, MRM mode). Calibration curves were used for quantification using MDMA-d5, THC-d3 and THC-COOH-d3 as internal standards. The method was fully validated according to international guidelines using neat oral fluid as well as oral fluid diluted with Cozart® DDS buffer.

Results: The assay was found to be selective for the compounds of interest. It was linear from 5 to 200 ng/mL for all analytes (R^2 <0.99). Extraction recoveries were typically > 70%. Accuracy, repeatability and intermediate precision were within the required limits using neat or diluted oral fluid. The limit of quantification was 5 ng/mL for all analytes, considerably below the recommended target concentration of the Australian Standard for oral fluid testing (AS 4760-2006). No instability was observed after repeated freezing and thawing. Using mobile phase for reconstitution, THC appeared to be unstable; however, further experiments have shown that the drug binds to autosampler vials which can be avoided by using methanol as a reconstitution solvent. The applicability of the assay was proven by analysis of authentic oral fluid samples from different roadside drug testing and workplace drug testing programs.

Conclusions: This fast, reliable and accurate extraction method combined with automated processing enables the detection and quantification of drugs of abuse in oral fluid.

Keywords: LC-MS-MS, oral fluid, drugs of abuse

P114. The incorporation of methamphetamine into hair

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Introduction: Methamphetamine is the most abused drug in our country. That's why forensic scientists have gotten a lot of questions and inquires on methamphetamine (MA) from police agency.

Aims: In this paper, we studied the incorporation of methamphetamine into hair in rat, how many times of administration are the critical points to detect MA in hair (to explore the detectability of a single administration of MA in hair), the difference of MA concentrations between black hair and gray hair, the MA concentrations in hair according to the amount and frequency of administration.

Methods: To investigate those things we used rats as animal models. The male rats were administered with doses of MA as follows: low MA doses (0.5 mg/kg body wt), medium doses (2 mg/kg body wt) and high doses (25 mg/kg body wt). The frequency rate of administrations is one time dose daily for 1, 2, 3, 4, 5, 15, 30 days. For the analysis of hair samples from the animals, the samples were extracted for 20 h in methanol containing 1% hydrochloric acid. The methanol/acid solution was evaporated to dryness and the resulting residue was derivatized with trifluoroacetic anhydride. Methamphetamine and and its metabolite, amphetamine were detected using selective ion monitoring (SIM) mode.

Results: In this study, methamphetamine in black hair from rats was detected after low, medium and high doses of administration in even single dose but when we considered the cut off criteria (0.5 ng/mg hair), the positive points were in low doses for 5 days, in medium doses for 3 days and in high doses for 3 days. The concentrations of methamphetamine in black hair from rats in low, medium and high doses for 15 and 30 were all more than the cutoff levels.

Conclusions: The presence of greater MA in black hair compared to MA in gray hair is consistent with many papers. MA and AM concentrations in hair from rats were found to be dependent on the amount and the frequency administered.

Keywords: methamphetamine in hair, GC/MS

P115. Analysis of abused drugs in oral fluid by GC/MS with automated SPE

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Introduction : Methamphetamine (MA) and cannabis are the most abused drugs in Korea. We have usually performed MA analysis in both urine and hair samples and cannabis analysis in urine samples only; however, MA and cannabis analysis in oral fluid is not established yet. Oral fluid is easy to collect/handle and can provide an indication for recent drug abuse.

Aims : In order to confirm the presence of MA, its main metabolite amphetamine (AM), Δ^9-tetrahydrocannabinol (THC) and its metabolite 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (c-THC) in oral fluid after screening with immunoassay an analytical method using automated solid phase extraction (SPE) and gas chromatography–mass spectrometry (GC-MS) was developed and fully validated. Also, the results in urine, hair and oral fluid from MA users and those in urine and oral fluid from cannabis users were compared.

Methods : Oral fluid specimens from 24 drug abuse suspects, submitted by the police, were collected using either Salivette® (Sarstedt, USA) or direct expectoration. The samples were screened with Fluorescence Polarization Immunoassay (TDxFLx, Abbott Co.) and microplate ELISA. For confirmation they were extracted using automated SPE (RapidTraceTM, Zymark, USA) with mixed-mode cation exchange cartridge (CLEAN

SCREEN®, 130 mg/3 ml, UCT), derivatized and analyzed by GC/MS using selective ion monitoring (SIM).

Results and Conclusions: The analytical methods using GC/MS were well established in oral fluid. The results from immunoassays were consistent with those from GC/MS. Even though large variations in the MA and AM concentrations were observed in three different specimens, oral fluid was considered as an alternative specimen for urine. However, a poor correlation was shown in the results of THC and c-THC between urine and oral fluid, which could be due to time intervals between drug use and sample collection.

P116. Quantification of cocaine in oral fluid by GC/MS-MS: results and statistical-based concentration intervals

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Introduction: Oral fluid tests are being widely used by governments in order to determine the consumption of drugs of abuse. A high number of studies, related to the legal framework, have been performed since 2007. These studies have been carried out in collaboration between local polices from Catalonia (collecting samples and carrying out road-site tests) and the IMLC laboratory (confirming results by more reliable techniques). The third step of the global study, with a minimal period of time of three years, consists in the statistical evaluation of results of drug consumption to establish quantitative intervals and relate them with the consumer profiles based on variables such as age, gender and fitness from the individuals. The final objective is to evaluate a real situation in order to promote legislative changes in a similar way as in other European countries.

Aim: This study pursues to establish a quantitative method to quantify cocaine in oral fluid samples with single consumption of cocaine collected in road controls. Moreover, fit quantitative results to a normal or Gaussian model by statistical methods, and afterwards establish concentration intervals in order to relate levels to age and sex.

Methods: The total number of samples includes 201 cases, which were taken from previously analysed samples, detected as positive for single consumption of cocaine by the immunoassay test and afterwards confirmed by GC/MS-MS. For GC-MS/MS analysis, sample preparation consists in the addition of 1 ml of oral fluid and 1 ml of phosphate buffer at pH 6 with 20 µL of cocaine-d3 of 10 µg/mL. Sample is homogenised for 10 min. and introduced in a Toxitube A® which is waved for 10 min., centrifuged and the organic phase is extracted, evaporated to dryness and reconstituted in 50 µl of ethyl acetate. 2 µL of the sample are injected in a split-less GC/MS/MS. A temperature program is carried out starting at 90 ºC for 1 min followed by a ramp of 20 ºC/min since 240 ºC and a second ramp of 5 ºC/min since 300 ºC by using a column VF-5ms 30 m × 0.25 mm. Detector used was a Varian ion trap with electron impact ionization considering the MS-MS alternative with the father ion 182 for cocaine. The limits of detection (LOD in ng/mL) and quantification (LOQ in ng/mL) respectively for the confirmatory analysis are 2.5 and 10.

Results: Quantitative results obtained from measurements carried out in oral fluid have been fitted to a Normal function by means of a Box-Cox transformation, leading to the function $X' = ([Coc]^{0.15}-1)/0.15$. Transformed concentration are found to correlate significantly ($p\text{-value}<0.05$) with age (according to Pearson test) but not with sex (according to Student t test). According to quantitative results, the following concentration intervals are defined: low concentration (LC): 0-5 ppm; medium concentration (MC): 5-100 ppm; high concentration (HC): 100-1000 ppm; and very high concentration (VHC): ≥ 1000 ppm.

Conclusion: Cocaine concentrations in oral fluid show a high degree of variability, which reflects the high number of factors that can have an effect on them. Besides the initial amount of cocaine consumed, the most important factors are the time elapsed since last consumption, the way of consumption (inhaled, smoked), chronic consumption and interferences due to extremely recent consumption. Results can be normalized with statistical significance. The intervals of concentration allow us to associate levels of cocaine with age and sex.

Keywords: GC/MS-MS, cocaine, oral fluid, statistics

P117. Respiratory depression after methadone ingestion and discovery of a polytoxicomania in a 10-year-old boy using segmental hair analysis

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Introduction: A 10-year-old boy was admitted in a pediatric intensive care unit after he was found by the emergency medical assistance service, at his mother's home, unconscious and with signs of severe respiratory depression and bradycardia. A toxicological screening of a urine sample collected at the boy's admittance was performed using immunological methods and revealed the presence of methadone and benzodiazepines. The laboratory consequently suggested to carry out a toxicological analysis on a hair sample in order to check the methadone-naïve status of the boy. A 6 cm long hair sample was taken and sent to the laboratory for a segmental analysis.

Material and Method: The hair sample was cut into 6 segments of about one cm long. After decontamination, each segment was hydrolyzed with 1 mL HCl 0.1N overnight at 56 °C. The hydrolysates were then neutralized by 1 mL NaOH 0.1N and extracted using a dichloromethane/diethylether (80/20) mixture in phosphate buffer pH 8.4. The dried extracts were reconstituted with 100 µL of mobile phase. The chromatographic separation was made by a UPLC system (Waters) equipped with an ACQUITY HSS C18 column in a gradient mode (ammonium formate/acetonitrile). The MS detector was a triple quadrupole detector (Waters) equipped with an ESI probe. Two different detection methods were applied on each extract: (1) a simple mass detection (MS) in full scan acquisition for the detection of a large number of molecules and (2) a MRM mode tandem mass (MS/MS) for a targeted and more sensitive detection of 157 molecules that are often found in medico-legal cases.

Results and discussion: The molecules detected in each hair segment are reported in the table below.

	Segment 1 (root)	Segment 2	Segment 3	Segment 4	Segment 5	Segment 6 (end)
Cocaïne	+	+	+	+	+	+
Benzoylecgonine	+	+	+	+	+	+
Ecgonine Methyl Ester	+	+	+	+	+	+
6 M.A.M.	+	+	+	+	+	+
Morphine	+	+	+	+	+	+
Codeine	+	+	+	+	+	+
Pholcodine	+	+	+	+	+	+
Methadone	+	+	+	+	+	+
EDDP	+	+	+	+	+	+
Nordiazepam	+	+	+	+	+	+
Prazepam	+	+	+	+	+	+
Citalopram	+	+	+	+	+	+
Acetaminophen	+	+	+	+	+	+
Levamisole	+	+	+	+	+	Not detected
Caffeine	+	+	+	+	+	+
Nicotine	+	+	+	+	+	Not detected

The segmental hair analysis (covering a 6-month period of exposure) revealed a continuous presence of methadone, benzodiazepines (prazepam and nordiazepam), an antidepressant (citalopram), acetaminophen, as well as narcotics (cocaïne and heroin). The levamisole, which is often used as a cutting agent for cocaïne, was also detected. The simultaneous presence of methadone and its metabolite (EDDP) is in favour of a regular contact/exposure during the last 6 months.

Conclusion: The segmental hair analysis allowed to demonstrate that the boy had been regularly exposed, during the 6 months prior to his hospital admission, to drugs and narcotics through passive or active exposure, consistant with a familial context of substitutive opioid treatment.

P118. LC/MS method for the determination of Δ^9-THC and 11-nor-Δ^9-THC acid in fly larvae of forensic toxicology interest

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Introduction: A careful examination of the colony of insects encountered on decomposing bodies, combined with the knowledge of insect biology, ecology and local environmental conditions can often provide valuable forensic insights. Furthermore, in recent years, an interest has also been directed towards the potential use of insects as alternative toxicological specimens in situations where, more traditional sources such as blood, urine, or solid tissues, are unavailable or not suitable for analysis. The information that can be provided by carrion-associated insects may lead not only to the estimation of time since death, but also to the detection of drugs or toxins. Cannabinoids are a group of compounds that originate exclusively from Cannabis sativa, the plant source of marijuana and hashish.

Aim and Methods: In cases of advanced decomposition, where the victim is suspected for cannabis abuse, the use of fly larvae feeding on the corpse might be important in the forensic investigation of death. The main active component of cannabinoids in both marijuana and hashish is Δ^9-terahydrocannabinol (Δ^9-THC). When Δ^9-THC enters the systemic circulation, it is metabolized to the liver in a series of metabolites, which the most important is 11-nor-Δ^9-tetrahydrocannabinoic acid (11-nor-Δ^9-THCCOOH). The use of cannabis is confirmed with the detection of these two substances. In this study a sensitive liquid chromatography-mass spectrometric (LC-MS) method was developed for the qualitative and quantitative determination of Δ^9-THC and 11-nor-Δ^9-THCCOOH in fly larvae belonging to the family Calliphoridae, which includes the most common species of insects found on decomposing remains in Greece. Δ^9-THC-D_3 and 11-nor-Δ^9-THCCOOH-D_3 were used as internal standards for Δ^9-THC and 11-nor-Δ^9-THCCOOH, respectively. For the ionization of the molecules an APCI ionization source in positive mode was used while the selected ions were monitored at 315 m/z and 345 m/z for Δ^9-THC and 11-nor-Δ^9-THCCOOH, respectively. The chromatographic isocratic separation was achieved on a Waters® symmetry C_{18} analytical column (2.1 × 150 mm, 5 µm) while the mobile phase was consisted of ACN/ammonium acetate 2 mM (70/30, v/v) running with a flow rate of 0.5 ml/min.

Results: The optimized method will be validated and used by our laboratory for the investigation of Forensic cases.

Conclusion: The development of this method will allow the simple and rapid determination of Δ^9-THC and 11-nor-Δ^9-THC acid in fly larvae of Forensic Toxicology interest.

Keywords: LS/MS, cannabinoids, fly larvae

P119. Violence under influence of methylphenidate documented by hair analysis

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Introduction: A 26 year old woman is accused of assaulting another female at a party on the 22nd November 2008. She states she was very drunk and was asked to leave the party. She became aggressive and damaged doors and cars and finally assaulted a female. She states her drink was spiked with Ritalin, which would account for her bizarre behaviour. No urine or blood taken as she did not mention this in her first interview with police. Hair was taken on 24th February 2009 and sent to the laboratory for methylphenidate testing.

Methylphenidate (Ritalin) is a phenethylamine derivative used in the treatment of childhood attention-deficit hyperactivity disorder (ADHD).

Methods: Hair strand was twice decontaminated using methylene chloride and then segmented. Each segment was cut into small pieces (< 1 mm). About 30 mg were incubated 3 hours in ultrasonic bath in buffer at pH 5.5, in the presence of 100 ng of MDMA-d_5 used as internal standard (IS). After neutralization with NaOH 1N, extraction with a mixture of hexane/ethylacetate (90/10) and evaporation to dryness, the residue was reconstituted in 200 µL of acetonitrile/formate buffer (5/95). Chromatography (LC-MS/MS) was achieved using a XTerra MS C18 column (100 × 2.1 mm, 3.5 µm) eluted with a gradient of acetonitrile and formate buffer delivered at a flow rate of 0.2 mL/min. A Thermo Ultra mass spectrometer was used for analyses. Ionization was achieved using electrospray in the positive ionization mode (ES+). For both methylphenidate and MDMA-d_5, detection was related to two daughter ions. (m/z 234>84 and 56 for methylphenidate; m/z 199>135 and 165 for IS).

Results: The method was linear from 0.5 to 500 pg/mg with a limit of quantification estimated at 0.5 pg/mg. As hair collection occurred 3 months after the event, we decided to analyse 3 sections of 4 cm. Methylphenidate was detected in the proximal segment at 1 pg/mg, while negative in the following sections.

Discussion: These results are in accordance with a single exposure to methylphenidate during the last 4 months (period including the party). This is a very low concentration in the view of what can be observed in hair of children under daily treatment (70 to 4170 pg/mg).

P120. Correlation of ethanol concentrations in whole blood and oral fluid collected with Statsure Saliva Sampler

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Introduction : Within the DRUID project, several epidemiological studies are currently being conducted in 13 European countries. Because of practical reasons, most countries choose to collect oral fluid with the Statsure Saliva Sampler in the road side studies and whole blood in the hospital studies. All blood and oral fluid samples collected from these drivers will be analysed for the presence of a list of minimum 23 psychotropic substances. Combination of the results from these studies should lead to an estimation of the relative risk of driving under influence. To be able to combine study results, a conversion factor between both sample types has to be defined for each substance.

Aim : The purpose of this study was to calculate the correlation of ethanol concentrations in whole blood and oral fluid collected with the Statsure Saliva Sampler and to define a conversion factor between the two sample types.

Methods : All samples were analysed using the Ethanol Gen.2 enzymatic method on a Roche Cobas Integra 400 system. Whole blood samples were first precipated using trichloroacetic acid. Oral fluid samples were analysed without prior sample preparation. Concentrations in oral fluid were volume-adjusted based on the weight of the collection devices after collection. The analytical cut-off used was 0.1 g/L, both for whole blood and oral fluid.

Results : Ethanol was present above cut-off values in samples from 145 drivers. Passing-Bablok regression plot (figure 1) shows a good correlation (r^2=0.9697) between the two sample types and a regression line with a slope of 1.14. The intercept of the regression curve does not significantly differ from 0 ($p<0.05$). The 95% confidence interval (CI- on the levels of agreement (LOA) in the Bland- and Altmanplot are -0.13 and +0.19 g/L.

Discussion : The current study suggests a slightly higher slope than a study by Jones *et al.*[1] but a narrower 95% CI on LOA. (Jones *et al.*: slope : 1.09 ; 95% CI on LOA : -0.18 and +0.25 g/L).

Conclusion : There is a good correlation between ethanol concentrations in oral fluid collected with Statsure Saliva Sampler and whole blood. A conversion factor of 1.14 (oral fluid/whole blood) can be used.

Reference: [1] Jones *et al.* Measuring ethanol in saliva with the QED enzymatic test device: comparison of results with blood- and breath-alcohol concentrations. (1995) J Anal Toxicol 19(3):169-74

Keywords: ethanol, oral fluid, whole blood, DRUID

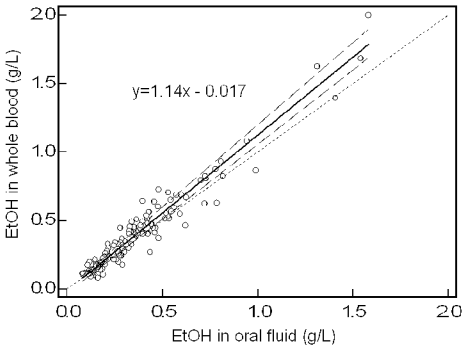

Figure 1: Correlation of ethanol in whole bloodand oral fluid (n=145).

P121. False positive results for phencyclidine on on-site oral fluid test Varian Oralab 6 caused by venlafaxine and o-desmethylvenlafaxine

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Introduction: A few case reports have demonstrated that the antidepressant venlafaxine and its main metabolite o-desmethylvenlafaxine can cause false positive results for phencyclidine (PCP) on qualitative immunoassays for urine testing. In a trial to test the reliability of the Varian Oralab 6 (an on-site test for screening of drugs of abuse in oral fluid), five positive screening results were found for PCP, although the prevalence of PCP is very low in Belgium.

Aim: The purpose of this study was to determine whether the positive results for PCP were true positives or if this could be explained by venlafaxine intake.

Methods: Oral fluid samples were spiked with venlafaxine and o-desmethylvenlafaxine (concentrations ranging between 100 and 2000 ng/mL) and applied to the on-site test to determine the cut-off for a false positive result.

A confirmation method for PCP, venlafaxine and o-desmethylvenlafaxine was developed on a ultra performance liquid chromatography system coupled to a tandem mass spectrometer (UPLC-MS/MS). PCP-d5 and trimipramine-d3 were used as internal standards. Oral fluid (100 µL) was extracted using liquid-liquid extraction with ethylacetate/heptane (4:1) after addition of ammonium bicarbonate buffer (0.2 M, pH 9.3). Detection was performed in multiple reaction monitoring mode using the following transitions: 244.3 > 158.9 and 116.8 for PCP, 249.3 > 164.0 for PCP-d5, 278.3 > 120.9 and 90.9 for venlafaxine, 264.3 > 106.9 and 152.9 for o-desmethylvenlafaxine and 298.3 > 102.93 for trimipramine-d3. Inaccuracy and imprecision were lower than 15%, and selectivity of the method was proven. This method was applied to four samples with a positive screening result; the fifth sample could not be analysed since there was not sufficient oral fluid left after the initial confirmation analysis.

Results: The tests with spiked oral fluid show that a false positive result is produced with the Oralab when concentrations of venlafaxine or o-desmethylvenlafaxine exceed 400-500 ng/mL. This corresponds to a normal therapeutic concentration [1]. No significant difference in cross-reactivity between the two compounds was observed. The confirmation analysis showed that there was no PCP present in the oral fluid samples. Venlafaxine and o-desmethylvenlafaxine were present in three out of four samples, respectively in concentrations of 523.8, 426.4, 717.0 and 98.7, 343.6, 474.9 ng/mL. In the fourth false positive case, no venlafaxine or o-desmethylvenlafaxine was found.

Conclusion : The experiments with spiked oral fluid clearly demonstrate that venlafaxine and o-desmethylvenlafaxine both can cause false positive results for PCP, even at therapeutic concentrations. Three out of four cases with a positive screening result for PCP were false positive due to this cross-reactivity. Screening results for PCP in oral fluid should therefore always be confirmed.

Reference: [1] de Castro *et al.* LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma. Study of correlation between venlafaxine concentrations in both matrices. Journal of Pharmaceutical and Biomedical Analysis 48 (2008): 183-193.

Keywords: phencyclidine, venlafaxine, oral fluid, cross-reactivity, LC-MS/MS

P122. Comparison of morphine, oxycodone, and fentanyl concenrations in oral fluid and plasma

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Introduction: Opioids are used as pain medication to treat cancer related pain of moderate or greater severity. Because drugs are absorbed differently in different patients, it is very important to monitor the level of opioids to ensure that sufficient amount has been transferred into blood circulation. Monitoring is usually done by measuring the drug concentrations in plasma samples. Collecting plasma samples can be very uncomfortable to the patient and acquires trained personnel. Finding an easy and non-invasive sample collection technique would enable routine monitoring of the drug concentrations that could be done for example at home. Oral fluid (OF), which is a filtrate of blood, is one possible sample matrix to be used instead of plasma. In order to estimate the drug concentrations in plasma, the OF/plasma ratios (OF/P) of the drugs have to be known. For morphine, there are many different OF/P ratios in the literature, values ranging from 0.4 to >100. The ratios for oxycodone and fentanyl have not been determined.

Aim: The aim of this study was to determine the concentration of morphine, oxycodone and fentanyl in plasma and OF of cancer patients in order to estimate the right dosage and route of administration of the drugs, and to calculate the OF/P ratios for these substances to see if OF could be used to monitor the opioid concentrations in plasma.

Methods: Samples were collected from cancer patients taking known doses of one of the three opioids studied as their main pain medication. Sample

collection was done when the cancer medication was in a stable state so that 5 $t_{½}$ had been passed since the medication started. The opioids were extracted from the samples with LLE and analysed with GC–MS.

Results: The OF/P for oxycodone ranged from 1.2 to 66.4 (mean 20.3, N=60), for morphine from 0.07 to 8.2 (mean 2.3, N=21), and for fentanyl from 1.2 to 10.8 (mean 3.6, N=7). There was some correlation in the concentrations of oxycodone and morphine between OF and plasma but due to the low number of fentanyl cases any reliable interpretations cannot yet be done from those results.

Conclusion: The OF/P ratio of oxycodone and fentanyl was always more than 1.0 and the concentration of oxycodone in OF in majority of the cases was a lot higher than that in plasma, which is an interesting finding. The OF/P ratio of morphine varied on both sides of 1.0, as has been noticed also in the previous studies. The study is still ongoing and the aim is to collect 5 OF and 5 plasma samples from each patient (N=20 patients/opioid). When all samples have been collected and analysed, more patient-specific data on opioid concentrations in plasma and OF will be presented.

Keywords: plasma, oral fluid, opioids, GC–MS

P123. Cocaine, benzoylecgonine and cocaethylene in hair of chewed coca leave by UPLC-DAD

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Introduction: The chewing ("coqueo") of cocaine leaves (*Erytroxillum coca*) is habitual in the NW and Argentine NE, having very peculiar sociocultural and religious connotations. Although the cases of abuse of the "coqueo" are not habitual in these communities, characteristic signs and symptoms have been reported. After an exhaustive bibliographical research, the authors have found few studies or data collections with respect to the presence in leaf of diverse cocaine related compound in pericraneal hair. Given the low levels of concentration that are possible to hope, a new method of liquid chromatography practiced, UPLC (ultra performance liquid chromatography), of extraordinary rapidity and resolution, due to the filling of column with particles <2 µ. The system was connected to a DAD detector. However, previously, hair samples were analyzed by radioimmunoassay (RIA) for the cocaine metabolite benzoylecgonine (BZE) in an attempt to verify a history of coca leave use.

Aim: The propose of our work was to study cocaine (C), benzoylecgonine (BZ) and cocaethylene (CE) in pericraneal hair of individual that chewing of cocaine leaves within or without simultaneous alcoholic beverages.

Material and Methods: Samples of pericraneal hair of 10 consumers of cocaine leaf were analyzed by RIA and after by UPLC-DAD. Initially, we washed the hair with dichloromethane and water to 37 ºC. The washing liquids were kept for later analysis. Next, the samples were processed with HCl 0.1 N to 50 ºC during 24 hours, and then filtrate. The water extract was processed by means of SPE (Bond Elut C-18), retaken with 500 µL of methanol. The extracts were injected in an equipment Aquity UPLC (Waters), with column C18 (50 × 2.1 mm id) with particle size of 1.8 µm. Mobile phase: potassium phosphate buffer, 20 mM, pH 7. DAD Detector 9695 Waters. LOD and LOQ of 0.1 ng/mg and 0.5 ng/mg.

Results: In three cases were detected BZ by UPLC-DAD (Rt: 3.12 m) and in two cases CE was detected (Rt: 3.92 m). BZ concentration was in the range: 3.7-30 ng/mg of hair and for CE: 0.4-1.2 ng/mg. However, in few cases were observed C signal and we couldn't quantify by UPLC-DAD (< LOQ). We will discuss possible incidence of the entrance route, type and/or variety of coca leaf consumed, in the cocaine detection in hair and origin of CE in chewing coca leave persons ("coqueros").

Conclusion: 1. [BZ] > [C]. 2. In case of simultaneous consumption of alcohol and coca leaves, it's possible to detect CE. 3. Its necessary to analyze other compound that be pattern of coca leave consume.

Keywords: chewing coca leave, cocaine, benzoilecgonine, cocaetilene, hair samples

P124. Analysis of drugs of abuse in oral fluid, urine and plasma

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Introduction: The biological matrix of choice in forensic toxicology is blood, but the interest in alternative matrices, such as the oral fluid, is always increasing. The use of HPLC-MS/MS is expected to fulfilling the needs of forensic analytical chemistry and forensic toxicology.

Aim: The aim of the research was to develop and validate a HPLC-MS/MS method for the determination of drugs of abuse (opiates, amphetamines, hallucinogens, cocaine, ephedrines, cannabinoids, togheter with their main metabolites) in different biological matrix, included saliva.

Methods: Sample preparation is extremely fast and easily applied: requiring a simple dilution of the matrix under examination with MeOH, centrifugation at 10000 rpm, and filtration with PTFE 0,45 µm membrane. Analysis in Liquid Chromatography is carried out using a 4,6 mm × 250 mm C18 column and acetonitrile and water, both containing formic acid 5 mM, are used for the mobile phase. As regards to quantitative determination, different acquisition periods were carried out in positive or negative ESI mode according to the chemical characteristics of the analytes, recording the ionic currents in Multi Reaction Monitoring. The majority of substances considered have basic characteristics, whereas carboxy-THC, the urinary metabolite of THC, only gives a response in ESI when working in negative mode. THC is detected in positive mode, albeit with slightly inferior instrumental response levels with respect to the other drugs examined. The choice of the periods proved to be fundamental, considering also the high number of substances analysed, and therefore, of the MRM transitions (two for every analyte) to which a minimum acquisition time (dwell time) are associated, which, altogether, would lead to an excessive scanning time with a poor definition of the chromatographic peaks. Moreover, the strategy enabled the insertion of at least one deuterated internal standard for each period.

Results: The performances of the analytical methods currently in use were assessed, be they screening or confirmation methods. From the real sample results, it was possible to verify, on the one hand, the modest specificity of the immunochemical methods, while on the other hand, the laboriousness of the analysis carried out in GC-MS, due to the necessity to purification and derivatization.

Conclusions: This new method proved to be simple, fast, robust, and sensitive and fully accomplishes the requisites in order to be used as a confirmation method in real samples.

Keywords: drugs of abuse, HPLC-MS/MS, oral fluid.

Clinical toxicology and therapeutic drug monitoring

P125. Development of a library-assisted toxicological screening method by LC-MS/MS

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Introduction: In clinical toxicology, a fast and specific method is necessary for the screening for different drug classes. In former time this has been done by GC-MS or HPLC with UV or diode-array detection, in recent years

the development of LC-MS/MS instruments and especially software tools enabled the use of LC-MS/MS in this respect.

Aims: The aim of the project is to develop a fully automated library-based screening system using LC-MS with online sample preparation.

Methods: In a first step, the established library contains more than 140 different substances. Due to different ionization properties of the compounds, 3 methods have been developed, which differ in the use of the buffers, in the mobile phase and the gradients applied. After solid-phase extraction of 2 ml urine, the different compounds were separated using HPLC with mobile phases consisting of acetonitrile, methanol, ammonium formiate buffer (pH 3.0) or ammonium acetate buffer (pH 4.0), respectively. After atmospheric pressure chemical ionization, the analytes have been detected by mass spectrometry using data-dependent acquisition. In order to estimate the sensitivity for the toxicological screening, the limits of detection were compared to estimated concentrations in urine (ECU) after therapeutic use of the drug. To test the performance of the method, > 100 patient urines have been analysed with the new library-assisted LC-MS/MS method, which have been previously screened by HPLC/UV and/or GC-MS. In a next step, the screening method is transferred to an automated online sample extraction system using turbulent flow chromatography to omit the solid phase extraction step. For the extraction, approx. 400 compounds out of the most important therapeutic classes have been tested on 7 different columns of different chemistries using various conditions.

Results: For the screening method with offline sample preparation, 20% of the > 140 substances which have been included in the library could be identified in a concentration in urine samples which corresponds to the ECU, about 70% even in a sometimes much lower concentration. For the patient samples, more than 95% of all formerly detected compounds could be reconfirmed by the new screening approach. Sometimes, in addition new drugs have been identified. As the amount of patient urine is restricted, occasionally less than 2 ml urine has been available for this comparison, leading to the conclusion that the rate of false negative results will be less if the amount of sample is sufficient. The evaluation of the extraction column gave the following result: over 96% of the tested compounds were extractable on an anion exchange column with a mobile phase adjusted to pH 8. Of the remaining compounds, 66% could be extracted using a polymer based column material. So, using both columns, 99% of all approx. 400 tested compounds can be extracted.

Conclusions: Library-based screening with LC-MS/MS seems to be an excellent addition to preexisting screening approaches. Especially the method using online sample preparation enables also a high-throughput for samples, which will get more and more important.

P126. Protective effect of *Nigella sativa* seeds extract on CCl4-induced hepatotoxicity in albino rats (toxicological, biochemical and histological studies)

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Introduction: Chronic liver diseases commonly result in liver fibrosis. Carbon tetrachloride (CCl4) is widely used for experimental induction of liver fibrosis. CCl4 is a potent hepatotoxin producing centrilobular necrosis which cause liven injury.

Aim: This study was carried out to investigate the role of *Nigella sativa* (NS) extract on the prevention of carbon tetrachloride (CCl4)-induced liver fibrosis in rats.

Material and Methods: Thirty adult male albino rats were used. They were divided into three groups: I-control group (10 rats) which subdivided into two groups (5 each) Ia that received only tap water and Ib that received *N. sativa* extract in a dose of 800 mg/kg orally every day for four weeks. II-CCl4-induced hepatotoxicity group (10 rats). The rats in this group received CCl4 in a dose of 0.15 ml /100 g body weight S.C for 3 days /week for four weeks III- heptotoxicity and *N. sativa* extract-treated group that received CCl4 in a dose of 0.15 ml /100 g body weight S.C for 3 days /week, in addition to extract of N. Sativa seeds 800 mg/kg orally every day for four weeks. After 4 weeks rats were scarified. Blood samples were collected. Plasma levels of aspartat transaminase (AST), alanine transaminase (ALT), and malondialdehyde (MDA) were determined by biochemical methods. Erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were also determined by biochemical methods. Liver was extracted. One portion used to analyze the levels of nuclear factor-κB (NF-κB) in liver tissue by western blotting. Another portion used for light and electron microscopic histological examination.

Results: CCl4-induced hepatotoxicity group showed significant rise in plasma AST, ALT, and MDA levels than control group while Heptotoxicity and *N. sativa* extract-treated group showed marked inhibition of all previous biochemical parameters. Also it was found that mean erythrocyte GSH-Px and SOD levels were found to be significantly lower in CCl4 induced group compared with the control one and levels of these parameters were found significant increases after NS treatment. The expression of NF-κB in the liver of hepatotoxicity and *N. sativa* extracted group were lower than that in CCl4-induced hepatotoxicity group. As regard histological changes, in CCl4-induced hepatotoxicity group liver showed moderate fatty degeneration and slight to moderate liver cirrhosis where as in heptotoxicity and *N. sativa* extract-treated group liver showed nearly similar to normal architecture pattern as confirmed by light and ultra structural examination.

Conclusion: Concomitant adminstration of CCl4 with NS seeds extract protects significantly the liver against CCl4 induced hepatotoxicity.

P127. Intoxication with brodifacoum – analytics and toxicokinetics

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Introduction: A 40-year-old man (185 cm, 92 kg) presented to an emergency department (ED) with nausea and increasing pains in his left upper abdomen. After aggravation of the symptoms and a body temperature of 39 °C he was instantly hospitalized. The CT-diagnostic revealed bleeding in the pelvis area and ileus of small intestine. Routine laboratory tests showed an INR of 3.67 (range: 0.85 – 1.15). Although the patient denied taking anticoagulants, the persistence of vitamin K-dependent factor deficiency led the medical doctors to investigate the serum for anticoagulant rodenticides.

Method: The analytical method used is suitable for the simultaneous identification and quantification of nine indirect anticoagulants in human plasma. It covers four superwarfarins (brodifacoum, difenacoum, difethialone, flocoumafen) and five other vitamin K antagonists (acenocoumarol, coumachlor, coumatetralyl, phenprocoumon, warfarin). The method is based on an acid (pH= 4.2) liquid-liquid-extraction followed by LC-MS-MS. Analytical separation was carried out using a Pursuit 5 PFP (150 × 30 mm, 5 µm). 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) pumped at a flow rate of 0.55 mL/min. The oven temperature was 40 °C and the injection volume was 25 µL. The lower limit of quantification (LOQ) is 0.010 mg/L (S/N >10).

Results: The coagulopathy was initially substituted with vitamin K1 (phytonadione). LC-MS-MS analysis confirmed a poisoning with brodifacoum (**B**, c_{max}=0.125 mg/L). Because the patient denied taking anticoagulants, this case presented clinical management problems. Under psychiatric

assessment and the confrontation with the analytical results the patient admitted to ingestion of rodenticide (500 g; 0,005% **B**) approx. 26 days prior presentation in a suicidal attempt. The analysis of further plasma samples resulted in a calculated elimination half-life of 30 days for **B**. The patient commenced an antidepressive and anxiolytic therapy with escitralopram. Further medications were metoprolol, hydrochlorothiazide and pantoprazol. After 16 days, the INR was 1.55 and the patient was discharged on vitamin K_1 20 mg orally daily and received psychotherapy.

Conclusions: This case emphasizes the need of a specific and sensitive analytical method if vitamin K anticoagulant poisoning is suspected and the patient show unexplained bleeding, abnormal INR values and response to treatment.

Keywords: intoxication, brodifacoum, vitamin K antagonists, LC-MS-MS

P128. Development and validation of a liquid-liquid extraction and gas chromatography/mass spectrometry methodology for cannabidiol and delta 9-tetrahydrocannabinol in human plasma

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Introduction: Cannabidiol (CBD), which represents approximately 40% of the cannabinoids found in *Cannabis sativa* plant, is devoid of typical cognitive and psychological effects of delta 9-tetrahydrocannabinol (THC), the main plant component. Studies with animals suggest that the CBD present anxiolytic properties, but very little tests have been done regarding clinical anxiety.

Aims: In the face of evidence of a cannabinoid system in humans and the growing interest in the therapeutic use of the CBD, the purpose of the present study was the development and validation of an analytical methodology for determination of CBD and delta 9-THC in human plasma.

Methods: The developed methodology is based on liquid-liquid extraction for the determination and quantification of CBD and THC in human plasma samples. Plasma samples (500 µl) were extracted with 2.5 ml of petroleum ether using shaker (30 min) and centrifugation (5 min, 2000 rpm). Then, after phase separation, 1.0 ml of organic phase was evaporated with N_2 (37 °C) and the residue reconstituted with 50 µl of acetonitrile. Extract was derivatized with 20 µl of BSTFA 1% TMC (45 min, 80 °C) and injected into a GC/MS.

Results: The method proved to be linear in concentration range from 5 to 500 ng/0.5 ml of plasma for CBD (R^2=0.9998) and 5 to 300 ng/0.5 ml (R^2=0.9895) for THC. The limits of detection and quantification were respectively 0.1 ng/0.5 ml and 0.5 ng/0.5 ml for CBD and 5 ng/0.5 ml and 10 ng/0,5 ml for THC. Values of inter and intra assay precision are respectively in the range from 5.5% to 12.7% and 2.1% to 8.1%. Values of inter and intra assay precision are respectively in the range of 1.2% to 12.0 and from 1.2 to 14.5 for CBD and THC. The efficiency of extraction was obtained in the range 54.6 to 93.2% recovery for the analytes.

Conclusions: The validated methodology using liquid-liquid extraction is rapid and simple, and was applied in a clinical study to correlate the dose after the controlled administration of CBD, in patients with social anxiety disorder. The results demonstrated adequate sensitivity and was suitable for application in clinical toxicology.

Keywords: cannabidiol, delta 9-tetrahydrocannabinol, plasma, gas chromatography/mass spectrometry, validation, anxiety

P129. Therapeutic drug monitoring of the new targeted anticancer agents imatinib, nilotinib, dasatinib, sunitinib, sorafenib and lapatinib by LC tandem mass spectrometry

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Introduction: The treatment of some cancer patients has shifted from traditional, non-specific cytotoxic chemotherapy to chronic treatment with molecular targeted therapies. Imatinib mesylate, a selective inhibitor of tyrosine kinases (TKIs) is the most prominent example of this new era and has opened the way to the development of several additional TKIs, including sunitinib, nilotinib, dasatinib, sorafenib and lapatinib, in the treatment of various hematological malignancies and solid tumors. All these agents are characterized by an important inter-individual pharmacokinetic variability, are at risk for drug interactions, and are not devoid of toxicity. Additionnally, they are administered for prolonged periods, anticipating the careful monitoring of their plasma exposure *via* therapeutic drug monitoring (TDM) to be an important component of patients follow-up.

Aim: The purpose of our work was to develop a liquid chromatography-tandem mass spectrometry method (LC-MS/MS) requiring 100 µL of plasma for the simultaneous determination of the 6 major TKIs currently in use.

Methods: Plasma is purified by protein precipitation and the supernatant is diluted in ammonium formate 20 mM (pH=4.0) 1:2. Reverse-phase chromatographic separation of TKIs is obtained using a gradient elution of 20 mM ammonium formate pH 2.2 and acetonitrile containing 1% formic acid, followed by rinsing and re-equilibration to the initial solvent composition up to 20 min. Analyte quantification, using matrix-matched calibration samples, is performed by electro-spray ionisation–triple quadrupole mass spectrometry by selected reaction monitoring detection using the positive mode.

Results: The method was validated according to FDA recommendations, including assessment of extraction yield, matrix effects variability (< 9.6%), overall process efficiency (87.1-104.2%), as well as TKIs short- and long-term stability in plasma. The method is precise (inter-day CV%: 1.3 - 9.4%), accurate (-9.2 to + 9.9%) and sensitive (lower limits of quantification comprised between 1 and 10 ng/mL).

Conclusion: This is the first broad-range LC-MS/MS assay covering the major currently in-use TKIs. It is an improvement over previous methods in terms of convenience (a single extraction procedure for 6 major TKIs, reducing significantly the analytical time), sensitivity, selectivity and throughput. It may contribute to filling the current knowledge gaps in the pharmacokinetics/pharmacodynamics relationships of the latest TKIs developed after imatinib and better define their therapeutic ranges in different patient populations in order to evaluate whether a systematic TDM-guided dose adjustment of these anticancer drugs could contribute to minimize the risk of major adverse reactions and to increase the probability of efficient, long lasting, therapeutic response.

Keywords: LC-MS/MS, anticancer targeted therapy, tyrosine kinase inhibitors, imatinib, nilotinib, dasatinib, sunitinib, sorafenib, lapatinib, plasma

P130. Certified solution standards and reagents for therapeutic drug monitoring applications

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Introduction: Highly pure, well-characterized, solution based standards or reagents are a good and efficient alternative to the use of neat materials in clinical, toxicology and therapeutic drug monitoring applications.

The accuracy, stability, and consistency of these materials are critical to ensure accuracy of results in the analytical laboratory, in clinical applications and medical device performance.

Aim: The aim of this poster is to demonstrate that certified solution standards and reagents offer a significant advantage over neat reference materials in terms of accuracy, consistency and stability. Long term stability of solution based materials is achievable when appropriate parameters are chosen in the design, preparation, packaging, and storage.

Methods: This poster presents the development and design of Cerilliant certified solution standards and reagents providing examples of pre-made solutions that exhibit multi-year stability. Factors critical to the selection of the analytes, diluents, storage and stability are discussed. These include: raw material handling, characterization and potency, certification and qualification of solutions, and homogeneity and stability of the solution. In addition factors impacting uncertainty are discussed including purity factor, weighing and preparation, and certification.

Results: Multiple examples are presented exhibiting long term stability of ampouled solutions for five or more years. Sealed containers and inert environment protect against evaporation and degradation. Analysis comparing preparation methods of certified ampouled solutions *vs.* neat reference materials demonstrate additional advantages of ampouled solutions including low risk of contamination, more efficient use of material, and improved consistency and accuracy.

Conclusions: High quality certified solution standards and reagents ensure accurate, consistent and reliable results and are an excellent alternative to the use of neat materials for clinical and toxicology applications and medical device performance. Certified reference standard solutions and reagents prepared in a diluent that promotes stability and packaged under argon in flame sealed ampoules can be stable for many years.

P131. β-glucuronidase-mediated reduction of oxazepam to nordiazepam

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Introduction: Many benzodiazepines are extensively metabolised, and then conjugated via glucuronidation in humans. β-glucuronidase is an enzyme often employed to de-conjugate β-glucuronides during urinary drug testing for benzodiazepines. It is commonly accepted that use of β-glucuronidase is a preferred method of hydrolysis over acid-catalysed hydrolysis, which is known to induce benzodiazepine degradation and transformation. Literature to date, however, has not reported any cases of benzodiazepine transformation initiated by β-glucuronidase.

Aim: The aim of this study was to identify benzodiazepine transformation products following various β-glucuronidase treatments on urine containing conjugated and un-conjugated (free) oxazepam.

Methods: Three β-glucuronidase enzymes were examined and were obtained from *Escherichia coli*, *Helix pomatia*, and *Patella vulgata*. The benzodiazepines investigated in this study were oxazepam and oxazepam glucuronide. After liquid-liquid extraction with dichloromethane/isopropanol (9:1), the extract was analysed by both LC-MS/MS in multiple reaction monitoring mode and GC-MS in selective ion monitoring mode for the presence of benzodiazepines. LC separation was achieved on an Alltima® C18 column (150 mm × 2.1-mm i.d., 5-µm particle size) with isocratic elution of a mobile phase consisting of 75% methanol and 25% water containing 5 mM ammonium acetate. GC separation was performed on a HP-5MS column (30 m × 0.25-mm i.d., 0.25-µm film thickness) following derivatisation of benzodiazepines with BSTFA (bistrimethylsilyltrifluoroacetamide) containing 1% TMCS (trimethylchlorosilane). Various incubation conditions were also examined.

Results: Incubation of β-glucuronidase with either oxazepam glucuronide in patient urine or free oxazepam added to blank urine resulted in the formation of nordiazepam. Nordiazepam formation was positively correlated with reaction temperature, incubation time, concentration of benzodiazepine used and enzyme concentration. Reducing capacity was observed for all three β-glucuronidase enzymes examined. When oxazepam (100 µg/mL) was incubated for 18 h at 52 ℃ with β-glucuronidases (1000 units/mL), nordiazepam was formed at a concentration of 78 ng/mL (with *E. coli* enzyme), 876 ng/mL (with *H. pomatia* enzyme), and 354 ng/mL (with *P. vulgata* enzyme) as measured by LC-MS/MS. GC-MS analyses of the TMS (trimethylsilyl) derivatives revealed comparable results.

Conclusion: Our study demonstrated conclusively that the novel reductive transformation pathway is directly attributable to the effect of β-glucuronidase treatment of urine. The reason for the different yields of reduced benzodiazepine by the three enzymes examined is not clear, because the reduction reaction mechanism by which β-glucuronidase is able to reduce oxazepam remains unknown. The findings of this study have both clinical and forensic implications and it is clear that the detection of nordiazepam in biological samples subjected to testing involving enzyme-catalysed hydrolysis should be interpreted with care.

Keywords: β-glucuronidase, oxazepam, nordiazepam, urine, reductive transformation

P132. An application of time-of-flight mass spectrometry (TOF-MS) drug screening for the multiple drug poisoning cases in critical care medical center

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Introduction: We have already demonstrated the exact mass database of 43 benzodiazepine using time-of-flight mass spectrometry coupled with liquid chromatography (TOF-MS). It is effective to be possible to identify the isomers which show a similar chromatographic elution, and are difficult to identify in conventional single LC-MS. However, many drugs other than benzodiazepines are contained in the sample of the multiple drug poisoning cases in a critical care center, Nippon Medical School (CCMC). Many of these drugs show the higher concentration than benzodiazepines in a biological sample. It is necessary to improve about the influence these drugs affect the benzodiazepine concentration. In addition to the benzodiazepines, the drug considered to be important for forensic toxicology was added, and a total of 142 kinds of exact mass databases were created. Exact mass database was applied to the sample of the multiple drug poisoning case of CCMC, and examined the discrimination of the TOF-MS drug screening.

Aim: The purpose of our work was to examine the discrimination of the TOF-MS drug screening in the multiple drug poisoning case of CCMC using exact mass database, in which 142 drugs were contained.

Methods: TOF-MS analysis was performed using 6520 Q-TOF (Agilent Technologies) equipped with a Zorbax C18 Extend column. Purine and fluorine compound solution was always introduced into the ion source, and real-time mass adjusting was performed. Specimens of serum, urine, and gastric contents were prepared utilizing the liquid-liquid extraction procedure with dichloromethane. Data acquired from TOF-MS measurements of 142 drugs as follows: barbiturates 10; benzodiazepines 32, metabolite of benzodiazepines 16; benzodiazepine antagonist 1; analgesics 5; muscle relaxant 1; antidepressant containing SSRI and SNRI 17; antipsychotics 20; non-benzodiazepine minor tranquilizer 2; antiepileptics 9; antiarrhythmics 1; hypotensor 3; sedative hypnotics 5; autonomic drug 1; hypertensor 1; Alzheimer therapeutic agent 1; Antiparkinson drug 3; metabolites 11, were used to create an exact mass database.

Results: Exact mass database was comprised molecular formulae, calculated exact masses, measured exact masses and retention times. Calibrations were also included in a database. Precision for the 34 drugs, which were required for analysis of the multiple drug poisoning case, was considered sufficient for quantitative analysis. In analysis of samples of multiple drug poisoning cases, who had taken ≥10 drugs, selectivity was improved using the TOF-MS exact mass database. The benzodiazepines which was showed microdose concentration was able to be distinguished and quantitated when it measured simultaneously with the drugs which were shown micrograms/ml level.

Conclusion: It was confirmed that the TOF-MS drug screening using a exact mass database is useful to simultaneous analysis of the multiple drug poisoning cases containing benzodiazepines.

Keywords: TOF-MS, exact mass database, drug, screening, patient

P133. Validation and long-term evaluation of a modified on-line chiral analytical method for therapeutic drug monitoring of *(R,S)*-methadone in clinical samples

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Introduction: Matrix effects, which represent an important issue in LC-MS or LC-MS/MS, should be closely assessed during method development. In the case of quantitative analysis, the use of a stable isotope-labelled internal standard (IS) with physico-chemical properties and ionization behaviour similar to the analyte is recommended [1].

Aim: To investigate the long-term stability of a previously developed method [2] used for chiral *(R,S)*-methadone (MTD) plasma quantification, after replacement of the initially used IS *(R,S)*-MTD-D9 by *(R,S)*-MTD-D3.

Method: After protein precipitation, the sample was injected into a column-switching system for on-line solid-phase extraction and LC-ESI-MS analysis. *(R,S)*-MTD-D9, which was chromatographically separated from *(R,S)*-MTD, was initially used as IS. Whereas no signal modifications were observed in regular situations, several samples presented *(S)*-MTD-D9 enhanced or suppressed signals, without a similar effect on *(S)*-MTD. Inversely, *(S)*-MTD signal enhancements were also observed in other cases, which were not corrected by *(S)*-MTD-D9. Considering these signal modifications, the initially used IS *(R,S)*-MTD-D9 was discarded and the co-eluting IS *(R,S)*-MTD-D3 was tested.

Results: Using the new IS, signal enhancements, which were attributed to short-term and long-term matrix effects, were still observed in some cases, but due to the similar chromatographic behavior of *(S)*-MTD and *(S)*-MTD-D3, these signal modifications were the same for the analyte and the IS. With this new condition, accurate results were obtained with quality control (QC) plasma samples analysed during complete method validation. A 1-year control chart process during 52 series of routine analysis was established using both intermediate precision standard deviation (SD) and FDA acceptance criteria. The results of routine QC samples were generally included in the ± 15% variability around the target value and mainly in the two SD interval illustrating the long-term stability of the method. It was found remarkable that on a 1-year follow up, the intermediate precision variability estimated during the validation was coherent with the QC variability during the routine use.

Conclusions: This study presents an illustration of potential interferences due to matrix effects that can be observed in LC-ESI-MS. The choice of an isotope-labelled IS that co-elutes with the target analyte appears useful in such cases. Using this approach, potential signal modifications should be the same for the analyte and the IS and should not compromise the quantitative result, as illustrated by the long-term accuracy of this method during routine therapeutic drug monitoring.

References : [1] Viswanathan CT *et al.* Pharm Res 2007; 24: 1962-73.
[2] Souverain S *et al.* Clin Chem Lab Med 2003; 41: 1615-21.

P134. Fast and simple GC-MS analysis for therapeutic drug monitoring of topiramate in human serum

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Introduction: Topiramate (2,3:4,5-bis-o-(1-methyl)-β-D-fructopyranose sulfamate) is a sulfamate-substituted monosaccharide, approved as antiepileptic drug (AED). The chemical structure is unrelated to any other anticonvulsant or mood regulating medication and the mechanism of action is unknown. The use of topiramate is indicated for the treatment of partial onset seizures and newly diagnosed epilepsy in adults, and as adjunctive therapy for primary generalized tonic-clonic seizures and Lennox-Gastaut syndrome. Concomitant treatment with enzyme-inducing AED's such as carbamazepine and phenytoin may require topiramate dose adjustment.

Aim: The aim of our work was to develop a method for the therapeutic drug monitoring of topiramate requiring only small sample volumes. Sample clean up needs to be robust and simple so the method can be easily implemented in daily clinical routine, yielding fast turn-around times.

Methods: Topiramate is extracted from 200 µL of serum by a simple liquid-liquid extraction using methaqualone as the internal standard. The extract is injected on a GC-MS in selected ion monitoring mode. Target and qualifier ions are 324 and 127, 171, 189 for topiramate and 235 and 132, 233, 250 for methaqualone.

Results: The assay was found to be linear in the concentration range 5 – 30 µg/mL with a mean coefficient of determination of 0.9985. Reported therapeutic concentrations range from 2 to 20 µg/mL and our LOQ is 1 µg/mL. Within-day and between-day precision were less than 10% at different concentration levels. The total run time is only 10 minutes. The developed method was used for the analysis of more than 800 real patient samples so far. These data will be presented with the concentration levels of other anti-epileptic drugs if used in combination therapy.

Conclusion: The developed method is very suitable for routine therapeutic drug monitoring of topiramate. The small sample volume needed, the simplicity and high throughput of the method make it particularly suitable for application in a clinical laboratory.

Keywords: topiramate, TDM, serum, GC/MS

P135. A semi-quantitative screening for the analysis of drugs of abuse and toxic compounds in serum/urine using ToxSpec™: an alternative to the BioRad REMEDI™-HS system

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Introduction: Screening of biological samples for drugs of abuse and other toxic compounds is one of the fastest growing markets in the forensic and clinical toxicology area. The main challenge is to provide rapid and accurate results despite the large number of potential analytes to be identified and the complexity of biological matrixes. A technique widely used in this area is HPLC combined with diode array detection (DAD) or UV detection; the most popular platform being the BioRad REMEDI™-HS. Unfortunately, this platform has recently been phased out of production leaving a significant

technological gap, which is rapidly being filled by newer and more effective HPLC-MS technologies.

Aim: We will evaluate here ToxSpec™ Analyzer, a new LC-MS system for screening and semi-quantitation of drugs and toxic compounds in serum and urine matrixes. It simplifies the workflow of assays while at the same time increasing confidence of identification in clinical toxicology.

Methods: Extraction procedure was performed using LLE (liquid/liquid extraction) with ToxiTube A (Varian, les Ulis, France). Serum and plasma samples were separated on a Hypersil Gold PFP™ 150 × 2.1 mm, 5 µm column. The screening was performed using an LXQ™ Ion Trap mass spectrometer utilising polarity switching, scan dependant tandem MS experiments and retention time windows were specified for each parent mass. The MS^2 spectra generated were processed through ToxID™ software.

Results: Currently, more than 150 patient samples (Serum and urine) have been analyzed using this screening method. The data have been compared on both instruments: the BioRad REMEDI™-HS LC/UV system and the ToxSpec™ LC/MS system. From these results, we observed that the ToxSpec™ Analyzer data are concurrent with the results obtained from the BioRad REMEDI™-HS system. Moreover, the ToxSpec™ system has identified additional compounds that were not detected with the BioRad REMEDI™-HS, due in most cases to a lack of sensitivity, specificity and co-elution issues. Secondly, a response factor for more than 100 molecules has been defined in order to perform semi-quantitation of the detected analytes.

Conclusion: The rapid and simple sample preparation, the ease of use of ToxID, the sensitivity and the specificity of the mass spectrometer, and a low cost per sample analysis make the ToxSpec™ Analyzer the appropriate replacement for the BioRad REMEDI™-HS system.

Keywords: LC-MS/MS, screening, serum, plasma, semi-quantitation

P136. Quantification of imatinib and nilotinib in serum by LC-MS/MS

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Background: Imatinib and nilotinib are inhibitors of the tyrosine kinase, which are widely used in the treatment of Philadelphia chromosome positive chronic leukaemia. Due to an additional interaction with PDGF, imatinib leads to oedemas in more than 50% of the patients receiving this drug. Both drugs are substrates of CYP3A4/5 and their metabolism can therefore be induced or inhibited by a variety of other drugs.

Aim: In order to perform clinical studies and to be able to quantify these drugs in situations of suspected overdose, an LC-MS/MS method has been developed.

Method: 10 µl serum was diluted with 50 µl water and then 100 µl of a methanol/acetonitrile (50/50) precipitation solution containing deuterated analogues of both drugs as internal standards have been added.

75 µl of the supernatant was removed after centrifugation and diluted with 75 µl of 20 mM ammonium carbonate buffer (pH 9.3). 30 µl have been injected into the LC-MS/MS. Separation has been performed on a C18 ODB uptisphere column (Interchim, Montluçon, France) using 5 mM ammonium carbonate buffer (pH 9.3), methanol and acetonitrile as mobile phase in a gradient mode. After atmospheric pressure chemical ionization (APCI) in the positive mode, the generated ions have been analysed by selected monitoring of m/z 494.4 → 394.2 for imatinib, 502.2 → 394.2 for imatinib-d8, 530.3 → 289.1 for nilotinib and 534.3 293.1 for nilotinib-d4, respectively with a LCQ Fleet mass spectrometer (Thermo Fisher Scientific, Reinach).

Results: The method has been fully validated. By analysing 5 different serum matrices of patients it has been proven to be free of ion suppression. The calibration curve was linear in the range of 0.025 to 5 mg/l, with a limit of quantification of 0.01 mg/l. The intra-day and inter-day imprecision of both analytes was < 12% and the respective accuracy in the range of 93 – 108%.

Conclusions: The method described allows the fast, precise and accurate quantitative determination of imatinib and nilotinib in serum of patients for example in the case of side effects due to suspected overdose. In addition, the method can and will be used for animal studies as the amount of serum needed is only 10 µl.

Keywords: imatinib, nilotinib, LC-MS/MS, serum

P138. Lack of adherence to long-term therapies as determined by TDM

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Introduction: One of the greatest problems with long-term therapies is adherence and this can only be determined through regular monitoring. For some drugs therapeutic drug monitoring (TDM) provides an easy mechanism of determining compliance. Historically TDM services were though to be over-used due to their convenience and this potential "misuse" needed to be offset against the clinical decision making support rendered. However, compliance continues to be a problem and under-monitoring has more potential adverse consequences than over-monitoring. With the increasing trend towards early discharge from hospital and greater emphasis on moving patient care and support services into the community, greater emphasis needs to be placed on regular TDM to support patient outcomes.

Aims: The aim of this study was to review the analytical data generated from TDM requests received by The West Midlands Toxicology Laboratory over a 9 year period. Only data originating from outpatient sources and on admission to emergency treatment units following therapeutic failures was selected. An audit was then undertaken to determine the frequency of compliance issues and to determine any trends in such findings over this period.

Methods: TDM analyses were performed using an Olympus AU400 automated platform utilizing Microgenics CEDIA® reagents. Daily assay control was ensured through the use of external reference material. Assays were re-calibrated according to the manufacturer's recommendations prior to use if control values exceeded ±2SD from their expected value. Data was collated following the routine TDM analysis for carbamazepine, digoxin, phenobarbital, phenytoin, and theophylline between January 2000 and December 2008.

Results: The greatest compliance issue, and risk of therapeutic failure, was associated with theophylline where an average of 78% of analytical results were outside of the target range The data for phenytoin, phenobarbital, digoxin and carbamazepine were 67%, 63%, 50% and 33% respectively. The data were remarkably consistent with no overall trend to improvement in compliance rates. On review of serial samples from individual patients, there could be seen repeated therapeutic failure interspersed with periods of stabilisation whilst in hospital.

Conclusions: Despite the ease of analysis, and cost effectiveness of routine TDM assays, there still appears to be a high proportion of results from the outpatient setting which indicate a risk of therapeutic failure. A more proactive strategy for monitoring long-term drug treatments needs to be developed to ensure patient safety.

P139. Monitoring of methadone plasma concentrations in participants of methadone maintenance programs

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Introduction: Methadone (MDN) is widely used in maintenance programs for opioid-dependent patients. The main metabolic pathway of MDN is N-demethylation to form 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

(EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). The dosage of MDN in maintenance programs could be up to 180 mg and the therapeutic plasma concentrations usually range from 50 to 1000 ng/mL with peak plasma levels at about 4 h after oral administration. AIMS: The aim of this study was the therapeutic monitoring of MDN and its main metabolites plasma levels in order to adjust MDN dosage in 19 patients. Determination of MDN and its metabolites in plasma is important in order to maintain plasma concentrations of MDN within an effective range.

Methods: A full validated GC/MS method was used for the determination of MDN, EDDP and EMDP in plasma samples obtained exactly before and 4h after MDN oral administration. Sample preparation included protein precipitation with acetonitrile and solid-phase extraction of the three analytes using Isolute Confirm HCX mixed-mode SPE columns.

Results: In cases of patients studied, MDN plasma concentrations exactly before (C_1) and 4h after MDN oral administration (C_2) ranged regardless of MDN dosage between 27.3 - 597.1 ng/mL and 94.0 - 932.9 ng/mL, respectively. Furthermore, ratio of MDN plasma concentrations (C_2/C_1) was found to be between 1.1 and 6.3. Some patients appeared withdrawal symptoms due to the fact that MDN can induce its own metabolism or because of interactions with other drugs (*e.g.* rifampicin).

Conclusions: Patients taking the same MDN dosage showed different plasma MDN concentrations possibly, due to inter-individual or interday differences in bioavailability and pharmacokinetic of MDN. Monitoring of MDN plasma concentrations is necessary for MDN dosage adjustment in order to obtain maximum treatment efficacy and to prevent toxicity, trying to maintain the ratio of MDN plasma concentrations 4 h after and exactly before the MDN administration > 2.

Keywords: methadone, methadone maintenance program, plasma, GC/MS

P140. A rapid and simultaneous analysis of psychopharmaceuticals in human body fluids with ultra-performanced liquid chromatography-tandem mass spectrometry

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Introduction: The overdose of antipsychotics and tranquilizers often causes intoxication, sometimes could be fatal. Thus various drugs need to be determined in the forensic toxicology, from many samples of suicidal or sexual assault cases. The ultra-performanced liquid chromatography (UPLC) system is a new applicable tool for rapid and sensitive analysis for determining many forensically important compounds by using a separation column with small particle (1.7 μm).

Aim: The purpose of this study is to analyze various groups of psychopharmaceuticals in the body fluids (or tissue samples) sensitively and simultaneously using UPLC-MS/MS system.

Methods: The mixture of twenty-five psychopharmaceuticals were spiked into 1 mL of human plasma and extracted by solid-phase extraction method generally used. The extracted compounds were dried and reconstituted with 125 μL of MS solvent. After filteration, 5 μL of the aliquots was injected to LC-MS/MS. The LC-MS/MS conditions were as follows; solvent used for analysis are; A (water / 0.1% formic acid) and solvent B (acetonitrile); gradient is programmed 80:20 to 5:95 (A:B) for 5 min; capillary and cone voltage are set at 3.0 kV and 50 V, respectively; collision gas for MRM analysis is Ar.

Results: All of analyzed compounds were sufficiently separated on the chromatogram within 2.5 min. The calibration curves for the compounds gave good linearity in the range of 1-1000 ng/mL. The detection limits of the psychoactive pharmaceuticals were estimated to be under 0.02 to 1 ng/mL, and the coefficient of variations of intra-day analysis for the compounds at 50 ng/mL were less than 12.5%.

Conclusion: UPLC-MS/MS analysis is useful for rapid and sensitive determination of psychopharmaceuticals from human body fluid. A standard LC-MS method needs more than 15 min for separating all of compounds while UPLC needs only 2.5 min. In many forensic cases, body fluids are often highly putrefied, and it is necessary to remove contaminants from samples. So we are now trying to detect psychopharmaceuticals and their metabolites from small amount of hair.

Keywords: ultra-performanced liquid chromatography, tandem mass spectrometry, psychopharmaceuticals, human body fluids

P141. SmileMS : a robust platform and library search algorithm for the large-scale identification of small molecules, using LC-MS/MS

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Introduction: Liquid chromatography tandem mass spectrometry (LC-MSMS) combined with library search is an emergent screening technique in clinical toxicology, forensic, and food testing. Today, and as a difference to GC-MS (EI spectra), the heterogeneity of the proposed instrumentation and acquisition methods leads to highly variable fragmentation patterns. Current dot-product based algorithms are insufficient to cope with this heterogeneity. There is therefore a strong need for a more robust approach. Furthermore, the growing community using this technique asks for possibilities to share libraries generated in each laboratory, which would allow access to a collaborative, community-wide mass spectral resource.

Aim: The main goal is to provide a robust and instrument independent LC-MSMS library search algorithm and a software platform, dedicated to small molecules. We also aim at providing spectral library sharing capabilities.

Methods: We developed and used X-Rank algorithm, a statistics-based algorithm, to score experimental fragmentation spectra with those of one or more libraries. SmileMS is a web-based client-server platform that uses X-Rank as its core algorithm. In order to test the cross-platform efficiency of the algorithm, data from different instruments including ESI-linear trap, ESI-3D-trap, GC-EI-MS have been searched against libraries generated from various instruments (NIST, Weinmann, others from collaborative labs). The applicability of the platform in a real large-scale analysis has been tested for a real case study on about thousand saliva samples and also compared with other tools. Metrics included interface usability and result quality.

Results: For all tested combinations (intra- and inter-instrument), X-Rank highlighted better specificity/sensitivity performances compared to MS-Search (NIST). For instance, considering a specificity of 95% X-Rank showed sensitivities up to 20% better than MS-Search for the LC-MSMS data and up to 80% better for EI spectra searched on linear trap instrumentation. On the saliva study, the comfortable user interface and the overall performance of the tool improved data interpretation time and confidence compared to Analyst 1.4.

Conclusion: Two different aspects of SmileMS have been explored and assessed. First, itsX-Rank algorithm provides good identification quality when fragmentation spectra are searched against a library created from the same instrument type as well as from a different one (cross-instrument use case). These performances are already today superior to those from all other tested software. Additional metrics will be explored to further enrich the

scoring model. Second, the ergonomy of the user interface and the rapidity of the platform have been identified as very useful for the forensic and clinical toxicology community

Current library sharing capabilities represent a promising add-on for forensic laboratories as well as for other applications and will also be improved.

Keywords: mass spectrometry, library search, clinical toxicology, forensic, identification

P142. Determination of fatty acid ethyl esters (FAEE) in meconium from newborn for detection of alcohol abuse during pregnancy in a maternal health evaluation study

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Introduction and aims: Excessive alcohol consumption during pregnancy is a serious hazard to the fetus and may lead to miscarriage, premature birth, increased peri- and neonatal mortality rate as well as retarded physical and mental development. Therefore, a new method for determination of fatty acid ethyl esters (FAEE) in meconium [1] was used for diagnosis of alcohol abuse in the last months of pregnancy.

Methods: About 1 g meconium was collected from newborn within the first 24 hours after birth and frozen at –80 °C for up 30 months until analysis. In one case the mother declared a continuous alcohol consumption of about two glasses of wine per day. For determination of ethyl myristate, ethyl palmitate, ethyl linolate, ethyl oleate and ethyl stearate, 50 mg meconium, each 25 ng of the d5-deuterated standards and 1 mL phosphate buffer (pH 7.6, 0.1 M) were placed into a 10 mL headspace vial, vortexed for 1 min, closed and analysed by headspace solid phase microextraction and gas chromatography-mass spectrometry. Each sample was analyzed twice. For interpretation, the concentration sum of the five esters C_{FAEE} was evaluated using a cut-off of 500 ng/g for alcohol abuse.

Results: In routine application, the method proved to be simple, robust, sensitive and reliable. The limits of determination found in the evaluation [1] were confirmed (20-50 ng/g for each ester) and were clearly below the cut-off of C_{FAEE}. In positive samples, ethyl palmitate, ethyl oleate, and ethyl linolate were the most abundant esters in quite different ratios, whereas ethyl myristate and ethyl stearate were found only in low concentrations. The difference between the two measurements of the samples was between 2 and 55% (mean 21%) of the mean value which is mainly due to inhomogeneous distribution of the esters within the samples. In the sample from the newborn child with the reported daily drinking of its mother of two glasses wine, C_{FAEE} =11,070 ng/g was measured. This is 22 times higher than the cut-off.

Conclusions: FAEEs in meconium proved to be a sensitive parameter for detection of alcohol abuse during pregnancy. In case of a reliable alcohol drinking report a highly positive C_{FAEE} result in meconium could be shown. For determining alcohol consumption in mothers with no or no credible drinking history further investigations are necessary to optimize the cut-off value in order to avoid false positive and false negative results. The method described here we used as well in the FRAMES-study (Franconia Maternal Health Evaluation Studies) where 606 samples of newborn children, born in the University hospital of Erlangen, were screened for FAEEs and correlated with additional data concerning lifestyle, health status and behaviour of the mothers. Results of this study will be published soon.

Reference: [1] Hutson JR, Aleksa K, Pragst F, Koren G., J Chromatogr B. 877 (2009) 8-12.

P143. Rapid determination of lithium in serum by capillary electrophoresis

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Introduction: The mood disorders, primarily major depressive disorders and bipolar affective disorders, are included in the greatest public health problems and are associated with significant reductions in productivity, health and longevity. After decades of use, lithium salts are still one of the most popular therapeutic approaches to the treatment of bipolar disorders, notwithstanding the introduction of new, less toxic drugs such as lamotrigine. Due to a narrow therapeutical range (recommended concentrations between 0.6-1.2 mM; toxic concentrations >1.4 mM), serum lithium concentrations must be strictly monitored during the treatment to avoid life-threatening neurotoxicity. For the purpose of therapeutical drug monitoring (TDM), lithium concentrations are currently measured in clinical laboratories by flame photometry or ion-selective electrodes. However, capillary electrophoresis (CE), already successfully applied to the analysis of inorganic ions in biological matrices, looks attractive for its easy application, low cost and high productivity and versatility. To the best of our knowledge, no reports have appeared in the literature on this promising application of CE.

Aim: the aim of the present work is to describe and validate an original CE method with indirect UV detection for the determination of lithium in serum samples, not requiring any sample pre-treatment but dilution with water.

Method: the used instrument was capillary electropherograph P/ACE MDQ (Beckman Coulter) with a DAD detector set at 214 nm operated in "indirect detection" mode. The separation was carried out in an uncoated fused-silica capillary (60 cm × 75 µm) under a constant voltage of 12 kV and 25 °C. The running buffer was composed of 10 mM imidazole, 15 mM α-hydroxybutyric acid (HIBA) and 7.5 mM 18 crown-6-ether, adjusted to pH 4.5 with acetic acid 1M. Serum samples were diluted 1:20 with water containing the internal standard (barium chloride) before injection.

Results: under the optimized above described conditions, a full validation of the method was carried out. LOD (0.1 mM), LOQ (0.3 mM), precision and accuracy (correlation with flame photometry) were suitable for the clinical aim of the method.

Conclusions: CE with indirect UV detection proved to be a valid alternative to current methods based on flame photometry and ion selective electrodes for lithium determination. In addition to greater versatility, CE looks to offer easy automation, lower costs and, conceivably, the direct determination of the "free ion", representing the active fraction of the drug.

Keywords: capillary electrophoresis, lithium, bipolar disorders

P144. Attempt to establish reference ranges for buprenorphine and norbuprenorphine in urine and serum samples from patients in substitution therapy

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Introduction: Buprenorphine (B), a partial µ-agonist opioid, is increasingly used for substitution therapy of heroin dependence. B is metabolized by dealkylation to norbupre-norphine (NB) pimarily by the cytochrome P450 isozyme CYP 3A4. Both B and NB are conjugated with glucuronic acid and subsequently excreted in urine. Little is known about the concentration of B and NB and their glucuronides under steady-state conditions in urine and serum samples from patients (pats.) in maintenance therapy. This however could be of importance when samples from presumptive non-compliant pats.

are to be analyzed. Compliance monitoring especially of pats. with take-home privilege should spot 1. pats. who pause from B, 2. pats. who spike B into their or a drug-free "friend-sample", 3. pats. who reduce dose, 4. pats. with additional B intake and 5. pats. who are not in steady-state.

Aim: The purpose of this study was to establish reference ranges for B and NB and their glucuronides in urine and serum and to propose "safe" criteria to verify patient compliance.

Methods: The drug assays and creatinine were performed on an Olympus AU640 according to the manufacturer (Microgenics). Total B and NB in urine was determined with GC/MS after enzymatic hydrolysis, SPE and acetylation. Free B and NB and their glucuronides in serum and urine were quantified with LC/MS after SPE. Compliant and stable pats. in steady-state (same dose since >14 days) with rare recent drug abuse were recruited by their physicians in Berlin, Erfurt, Halle and Stockholm (n = 54; 35 male, 19 female). The B doses were divided into four groups: 14 pats. 0.4-5.2 mg B/d, 14 pats. 6-10 mg B/d, 16 pats. 12-16 mg B/d and 10 pats. 20-32 mg B/d. 33 pats. had the take-home privilege. Paired urine and serum samples were taken twice with 10-14 days in between (urine: n = 108, serum: n = 107).

Results: Urine drug screening gave significantly lower positive rates when compared to an unselected patient population supporting that stable patients were selected. The B-CEDIA showed equimolar crossreactivity for B and B-glucuronide when tested with the corresponding reference material and revealed a good correlation to total urinary B from GC/MS and to the sum of urinary free B + B-gluc from LC/MS. The concentration of total and free B and NB in urine (corrected for creatinine) and serum showed only a weak correlation with dose. However the molar ratio of total B/NB seemed promising: there was no correlation with dose and 90% of the samples were within 0.08-0.67. For the individual patient the mean difference between his two values was only 24% (sd = 16). For 90% of the samples the molar ratio of urinary free B/B-glucuronide was 0.0085 to 0.120 and 0.120 to 0.642 for the free NB/NB-glucuronide ratio, indicating a higher conjugation rate for B. In serum these ratios were 0.36 to 6.45 and 0.11 to 1.50 resp. indicating a higher proportion of free B. The mean difference between the two patient values of free B was 27% (sd = 22) making this analyte an additional candidate for compliance monitoring.

Conclusion: The hypothesis is put forward that a ratio of urinary total B/NB >1.0 possibly results from non-compliance or proves that the patient has not reached steady-state.

Keywords: buprenorphine, norbuprenorphine, glucuronides, serum, urine

P145. Quantification of recent antidepressants from micro whole blood samples collected on filter-paper by fast gas chromatography coupled with tandem mass spectrometry

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Introduction: Since 1960, the filter paper 903 S&S produced by Whatman has been widely used as support for collecting newborns' capillary blood for the screening of neonatal metabolic disorders. Called dried blood spots (DBS), this procedure offers the advantage of being less invasive and more cost effective in terms of sample collection, shipment and storage as compared to venipuncture. Recently the DBS sampling has been applied to the monitoring of pharmaceutical compounds including antimalarials, antiepileptics, antiretrovirals, antibiotics, and immunosuppressive drugs.

Aim: Due to the advantages of the filter-paper as a support for the collect, shipment and storage of biological samples, an analytical procedure was developed and validated for the quantification of selective serotonin reuptake inhibitors (SSRI, Fluoxetine as model compound) from 10 µl whole blood spotted on Whatman 903 filter-paper.

Methods: Before analysis, DBS were punched out and antidepressants were simultaneously extracted and derivatized in a single step by triethylamine 0.02% in butyl chloride and pentafluoropropionic anhydrous (PFPA) for 30 min at 60 °C under ultrasonication. Then derivative analytes are separated on a gas chromatograph coupled with a mass spectrometric triple quadrupole operating in the selected reaction monitoring mode after negative-ion chemical ionization (GC-NCI-MS/MS) with a total run of 6 min.

Results: To establish the validity of the method to internationally accepted criteria, accuracy, precision and selectivity were carried out. The assay was found to be linear from 20 to 500 ng/mL for all analytes. Despite the use of a small sampling volume, the limit of quantification (LOQ) and detection (LOD) were 20 pg/mL and 2 pg/mL respectively, and the repeatability was found less than 15% for all concentrations tested. Short-term stability of DBS was evaluated at -20 °C, 4 °C, 25 °C and 40 °C up to 30 days. Finally, the method was applied to real cases.

Conclusion: This validated DBS method combines an extractive-derivative single step with a fast and sensitive GC-NCI-MS/MS technique. With a micro volume of blood sample, the procedure offers a patient friendly tool in many analytical fields such as treatment adherence control, therapeutic drug monitoring or toxicological analyses.

Keywords: fast GC, NICI-MS/MS, antidepressants, whole blood, dried blood spots, therapeutic drug monitoring, forensic toxicology

P146. Acute respiratory failure after exposure to limonene: a case report

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Introduction: D-limonene (4-isopropenyl-1-methylcyclohexene) is the chemical name for orange oil. It's a renewable resource and is a by-product of orange juice manufacturing. Orange oil is used in cleaning solutions, pet shampoos, soaps and perfumes. Limonene and its oxidation products are skin and respiratory irritants. Acute exposure to D-limonene has rarely been reported in deaths.

Aim: The purpose of our work was to demonstrate unusual toxicological cause of respiratory failure and recommend full toxicological screening tests especially in suspicious cases.

Case: In this case report, we present a case of a previously healthy 30-year-old man who presented to the emergency department with acute respiratory failure. Non-toxicological causes were excluded. After history taking, clinical examination and toxicological analysis using GC/MS, shimadzu 2010, splitless mode of injection.

Column temperature: 170 °C for 2 minutes and then programmed at 16 °C per minute to 270 °C and held for 8 minutes. The case was diagnosed as toxic exposure to limonene dissolved in organic solvent. The patient was exposed to limonene for many years in air fresheners. In spite of supportive treatment, the patient did not survive. The clinical and laboratory findings are discussed.

Conclusion: Limonene and its oxidation products are skin and respiratory irritants. Inhalation of these chemicals carries the risk of toxicity, which could be missed in diagnosis and hence treatment. This should encourage physicians working in emergency units to analysis for all available chemicals to avoid misdiagnosis.

Keywords: GC/MS, limonene, chemical toxicity

P147. Quantitative method implementing fast polarity switching quadrupole mass spectrometry for analysis of ten anti-epileptic drugs in plasma samples

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Novel Aspect/Aim: Demonstrates critical benefits of very fast polarity switching in screening for anti-epileptic drugs in clinical research and forensic applications.

Introduction and Objective: Main objective of this study is to present a simple and reliable LC-MS method for the determination of older and newer anti-epileptic drugs employing fast positive and negative polarity switching (less than 25 ms). Collecting both positive and negative data in a single experiment allows for efficient drug identification as well as it eliminates a need for two separate polarity experiments resulting in great reduction of instrument time and solvent usage.

Method: The need for a faster polarity switching experiments without sacrificing duty cycle time is crucial in applications quantifying panels of positively and negatively ionized compounds. Method quantifying 10 antiepileptic drugs advantaged from 25 ms polarity switch in producing high quality quantitative data.

The method was developed using an Accela high speed LC system with a 50 × 2.1 mm 5 µm Hypersil GOLD C18 and a TSQ Access MAX triple-quadrupole mass spectrometer. Detection was achieved by atmospheric pressure chemical ionization in SRM mode with constant positive/negative polarity switching. A six-minute LC method with gradient using methanol and water, at a flow rate of 400 µL/min was created for the rapid separation. Plasma samples were processed with protein precipitation method.

Results/Conclusions: The quantification of 10 anti-epileptic drugs positively ionized: lamotrigine, levetirocetam, gabapentin, oxcarbazepine, carbamazepine, primidone and tiagabine. Negatively ionized: zonisamide, Phenytoin, Topiramate) on triple-quadruple mass spectrometer was performed in efficient 6-minute method with LOQ of 0.5 µg/mL. The developed method successfully quantifies ten antiepileptic drugs in a single experiment with simultaneous positive and negative polarity switching. The switching time is less than 25 ms which improves the duty cycle time and more scans across the peak are collected resulting in improved data precision. Method was validated by processing and analyzing plasma QC samples prepared with concentrations across method calibration range (0.5-50 µg/mL). The samples were analyzed in 5 replicates in 3 different batches to obtain inter and intra assay variability.

P148. An on-line extraction LC-MS method for screening and quantification of multiple therapeutic drugs in human urine

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Novel Aspect/Aim: Fast and reliable on-line sample preparation TurboFlow® LC-MS method was developed to screen and quantify different therapeutic 31 drugs.

Introduction: Patients that are on therapeutic drugs including antidepressants, hypnotics, stimulant, cardiac, antihistamins and some other drugs must stop taking those drugs before certain medical procedures. A low level of the drug in the urine must be confirmed with an analytical method. On-line sample preparation TurboFlow® LC-MS method was developed to screen and quantify 31 drugs from different therapeutic classes.

Method: Urine samples were diluted with methanol containing deuterated internal standards, centrifuged and injected onto Cyclone MAX turbulent flow preparatory column followed by Hypersil GOLD PFP analytical column. The method acquisition time was 15 min. MS detector was set to collect two SRM transitions per each analyte. Ion ratio and retention time were used for compounds identification. Method was implemented on Aria™ Turbo Flow system and Quantum Access™ MS detector.

Method Validation: The urine calibrators were prepared in house by spiking blank urine with analytes to concentrations of 1, 10,100 and 1000 ng/mL. QC samples in concentrations across calibration range were prepared to validate method accuracy and precision.

Results/Conclusions: The quantification was performed with LOQ of 1 ng/mL for 24 analytes, 10 ng/mL for 2 analytes, and 100 ng/mL for 2 analytes. LOQ for Nicotine was 1000 ng/mL. Calibration linear ranges from LOQ concentration to concentration of 1000 ng/mL were obtained for all but 1 analyte. Validation data demonstrates robust, efficient on line extraction LC-MS method with a linearity range, accuracy and precision meeting industry standards.

P149. Simultaneous quantitative determination of opioid dependency treatment drugs in human urine using UPLC®-MS/MS

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Aim: To develop and validate a single, simple and rapid UPLC-MS/MS method for the quantitative determination of the opioid dependency treatment drugs methadone, buprenorphine and dihydrocodeine, in human urine.

Methods: Quantitative analysis was performed on methadone (METH), buprenorphine (BUP) and dihydrocodeine (DHC) and two drug metabolites: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and norbuprenorphine (NBUP) with five internal standards: methadone-D9; buprenorphine-D4; dihydrocodeine-D3; 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine-D3; norbuprenorphine-D3; dihydrocodeine-D6. All authentic urine samples, calibrators and quality controls (QCs) were prepared by enzymatic hydrolysis followed by a simple liquid/liquid extraction procedure. Chromatography was achieved using a Waters Acquity UPLC® system. Analytes were separated on a Waters Acquity UPLC HSS T3 (2.1 × 100 mm, 1.8 µm) column using a gradient elution over 5 min with a mixture of 5 mM ammonium acetate containing 0.025% formic acid in water (A) and methanol (B). A Waters TQD mass spectrometer was used for analysis with electrospray ionisation in positive mode (ESI+). Two MRM transitions were monitored for each compound and each transition was optimised to achieve maximum sensitivity.

Results: Responses were linear for all compounds over the investigated range *i.e.,* 25-25000 ng/mL for METH and EDDP, 25-2500 ng/mL for DHC, 2.5-250 ng/mL for BUP and NBUP. Precision (intra and inter-assay) and accuracy were good with CV's for spiked QC samples <14% and 96-115%, respectively. The use of the liquid/liquid extraction was demonstrated to be very efficient and gave reproducible recoveries *i.e.,* >84% for all analytes. All compounds were shown to be stable in extracted samples over 24 hours. Limits of detection were 0.5 ng/mL for EDDP, 1 ng/mL for METH & DHC and 2 ng/mL for BUP & NBUP. Matrix effects were assessed by spiking blank extracted patient samples (n=7) with all compounds and comparing the responses against the equivalent concentration for a solvent standard solution. Average matrix effects were found to be acceptable for METH, EDDP & DHC. More significant effects were observed for BUP & NBUP (-39% and +19.3%, respectively), consequently deuterated internal standards were included throughout to minimise the impact of the matrix on data quality. The method was applied to the analysis of clinical patient samples (n=58) for METH, EDDP, BUP & NBUP which were previously analysed by two separate, established and validated HPLC-MS/MS methods; all samples showed good agreement. No suitably quantified patient samples could be obtained for DHC, however samples containing DHC (n=20) that had been qualitatively analysed using thin layer chromatography were obtained and analysed using the newly developed method. The qualitative results showed good correlation.

Conclusion: The developed method provides a simple, sensitive and robust solution for the quantitation of opioid dependency treatment drugs in human urine. The use of a single assay, suitable for the simultaneous analysis of all of the drugs of interest, alleviates the issues involved in switching between methodologies for the different drugs, offers significant time-saving benefits and a fifty percent reduction in sample preparation time.

Keywords: buprenorphine, methadone, UPLC

P150. Development of highly sensitive antibodies for the detection of tricyclic antidepressants, trazodone and related compounds

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Introduction. Tricyclic antidepressants (TCAs) are indicated for the treatment of clinical depression, neuropathic pain, nocturnal enuresis and ADHD. Whilst therapeutic drug monitoring (TDM) of TCAs is well established in the treatment of depression, TCA overdose remains the most common cause of death from prescription drugs. Trazodone is used in the treatment of depression, panic attacks, and agoraphobia, as well as insomnia, aggressive behaviour and cocaine withdrawal. Studies have shown that in human liver, this compound is metabolised to m-chlorophenylpiperazine (mCPP). mCPP is also an intermediate in the production of nefazodone, etoperidone and mepiprazole. For TDM and toxicological applications, the use of specific, sensitive and rapid immunoassays enabling determination of these compounds in biological fluids is advantageous.

Aims. We report the development of a highly sensitive monoclonal antibody presenting a broad specificity profile against TCAs and two highly sensitive polyclonal antibodies with different specificity profiles for trazodone, mCPP and nefazodone. This is of value for the development of immunoassays for the screening of these compounds.

Methods. Monoclonal antibody against TCAs: sheep were immunized with nortryptyline conjugated to bovine thyroglobulin (BTG) as a carrier. Lymphocytes were then collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of generic TCA antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. Polyclonal antibodies against trazodone and related compounds: trazodone and mCPP were coupled by way of a crosslinker to bovine thyroglobulin (BTG) as carrier. The resulting immunogens were administered separately to adult sheep on a monthly basis to provide target-specific polyclonal antisera. IgG was extracted from the antisera and evaluated via competitive ELISA.

Results. Initial evaluation of the monoclonal antibody against TCAs showed significant generic recognition for a wide range of TCAs and metabolites (%cross-reactivity ranging 20-700 relative to 100% dothepin). Sensitivity values, expressed as IC50 were <1 ng/ml (nortriptyline, protriptyline, cyclobenzapine and trimipramine); <3.5 ng/ml (lofepramine, dothepin, doxepin, imipramine, chlorpromazine, amitriptyline, desipramine, norclomipramine, promazine and norchlorpromazine); 9.94 ng/ml for 2-hydroxyimipramine and 14.13 ng/ml for nordoxepin HCl. The polyclonal antibodies against trazodone and related compounds revealed different specificity profiles. One antibody was specific for trazodone (%cross-reactivity 100) without recognition of mCPP and nefazodone with IC50 of <0.15 ng/ml for the target molecule. The other antibody exhibited a broader specificity profile with recognition of mCPP, nefazodone and trazodone (%cross-reactivity 100, 217.3 and 1593 respectively relative to mCPP) and IC50 <8.5 ng/ml for mCPP, <0.6 ng/ml for trazodone and <4.0 ng/ml for nefazodone.

Conclusions. The development of the monoclonal antibody exhibiting high sensitivity and specificity for a wide range of TCAs and metabolites, as well as the development of two highly sensitive polyclonal antibodies against trazodone and related compounds with different specificity is valuable for use in developing more effective immunoassays applicable to TDM and toxicology fields.

P151. The application of simultaneous determination of valproic acid and its three metabolites (2-ene-VPA, 4-ene-VPA, 3-keto-VPA) in serum by GC-EI-MS in acute poisoning

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Introduction: Valproic acid (VPA; 2-n-propylpentanoic acid) is a simple branched-chain carboxylic acid but extensively metabolized by the liver to over 50 known metabolites possessing anticonvulsant activity and toxic effects. VPA undergoes complexity metabolism via direct glucuronization, mitochondrial β-oxidation and cytosolic ω- and ω_1-oxidation. The mitochondrial β oxidation is most important metabolic route of VPA provide 2-propyl-2-pentenoic acid (2-ene-VPA) with antiepileptic activity at therapeutic level and 3-oxo-2-propylpentanoic acid (3-keto-VPA), the main metabolite presents in blood. Minor pathways include cytochrome P450-catalysed oxidation via desaturation leads to a hepatotoxic derivative, 2-propyl-4-pentenoic acid (4-ene-VPA).

Aims: The aim of this study was to develop a highly sensitive and selective GC-EI-MS analytical method of simultaneously determination of valproic acid and its three metabolites (2-ene-VPA, 3-keto-VPA and 4-ene-VPA) in human serum. The validated method was used in evaluation of metabolic profiling of valproic acid in serum acute poisoned patients.

Methods: To separate and measure serum concentration of valproic acid, 2-ene-VPA, 3-keto-VPA and 4-ene-VPA, a specific method (GC-EI-MS) was used. Quantitation was achieved by the addition of deuterated valproic acid as internal standard (IS). Limits of quantitation (LOQ) were 1.0, 0.1, 0.1 and 1 μg/ml for VPA, 2-ene-VPA, 4-ene-VPA and 3-keto-VPA, respectively, with linearity ranging up to 10 μg/ml for 4-ene-VPA and 2-ene-VPA, and 30 μg/ml for 3-keto-VPA, and 100 μg/ml for VPA. Intra-assay accuracy and precision ranged from 0.2 to 8.8% and 0.8 to 9.3%, respectively. Inter-assay accuracy and precision ranged from 0.5 to 4.1% and 1.9 to 4.2%, respectively.

Results: Our study includes four poisoned patients, who ingested from 12 to 46 g of valproic acid. At admission (one to 29 hours since intoxication), the measured serum concentrations of valproic acid were elevated to the following toxic levels: 150.6, 260.4, 338.7 and 430.2 μg/ml. Simultaneously, the determined serum concentrations of 4-ene-VPA, 2-ene-VPA and 3-keto-VPA were ranged from 0.1 to 1.3 μg/ml, 1.1 to 3.4 μg/ml and 1.4 to 5.9 μg/ml, respectively.

Conclusions: Mitochondrial β-oxidation was probably impaired in the acute valproic acid intoxication result in decrease 2-ene-VPA and 3-keto-VPA concentrations. In consequence valproic acid metabolism switched to alternate (minor) metabolic routes leading to elevated 4-ene-VPA concentration. Elimination of valproic acid may be lengthen in comparison to VPA therapeutic use.

P152. Determination of 14 atypical antipsychotics, antidepressants and metabolites in plasma and whole blood by UPLC-MS-MS

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Introduction : Atypical antipsychotics are used for the treatment of mental disorders like schizophrenia. Antidepressants are used to treat mood disorders such as depression. They are both among the most commonly prescribed drugs. They are of interest in clinical toxicology for therapeutic

drug monitoring and in forensic toxicology because of their involvement in suspect deaths.

Aim: The aim of this work was to develop and validate a rapid, specific and sensitive UPLC-MS-MS method for the quantitative determination of bupropion, hydroxybupropion, clozapine, desmethylclozapine, loxapine, amoxapine, mirtazapine, moclobemide, olanzapine, pimozide, quetiapine, risperidone, 9-hydroxyrisperidone and trazodone in plasma and whole blood. The method is intended to be used in routine applications for both clinical (plasma) and forensic (whole blood) toxicology.

Method : Analytes were extracted from plasma or whole blood by liquid-liquid extraction with hexane:ether 1:1, at alcaline pH. Four deuterated analogues were use as internal standards to accomodate the particularities of some analytes and the various chemical structures. The extract were evaporated, reconstituted in mobile phase and analysed by UPLC-MS-MS. Chromatogaphic separation was performed on a Waters Acquity UPLC BEH C_{18} (2.1 × 50 mm, 1.7 µm) column with a flow rate of 0.5 mL/min. The mobile phase consisted of a gradient of methanol and formic acid 0.1%. A Quattro Premier XE tandem mass spectrometer (Waters) was used with electrospray ionisation in positive mode and operated in the MRM mode. The total run time was less than 5 minutes per sample.

Results : The UPLC separation provided high resolution with peak width of about 5 seconds for all compounds. The high efficiency, combined with the high linear velocity of the mobile phase resulted in a fast chromatographic run, allowing a higher throughput than traditionnal HPLC. The method showed good sensitivity with LOQs between 1 µg/L and 4 µg/L. The extraction recoveries were between 77% and 99%. No significative matrix effect has been observed. The method is fully validated under ISO 17025.

Conclusion : The method described here allows specific identification and quantitation of 14 atypical antipsychtotics, antidepressants and some of their metabolites in plasma and whole blood. The method is suitable for clinical and forensic applications.

Keywords: antipsychotics, antidepressants, UPLC-MS-MS

Chemometry and method validation

P153. The profiling of ecstasy tablets using physical characteristics and organic impurities as sources of information

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Introduction: When ecstasy tablets are seized in Switzerland, the first legal step is to identify the illicit active substance(s). Beyond this legal requirement, other information extracted from the seizures (*i.e.* physical characteristics and chemical composition of the tablets) are very valuable to gain intelligence on drug production and trafficking and may help ongoing police investigations. In fact, drug profiling provides means to highlight physical or chemical links between different drug seizures.

Aims: This work evaluates the added value of organic impurities for the profiling of seized 3,4-methylenedioxymethamphetamine (MDMA) tablets previously classified using their physical features. On one hand, physical characteristics are produced by the tabletting process and yield preliminary profiling data that may be valuable in a first stage for investigation purposes. On the other hand, organic impurities are produced during the MDMA synthesis and are generally considered to bring more reliable information, particularly for presentation evidence in the court.

Methods: 120 seizures made by the police were first classified according to their physical characteristics (mainly weight, thickness, diameter and the presence of a score on the surface of the tablets). Then all samples were analysed using a harmonised gas chromatography / mass spectrometry (GC/MS) method. In order to compare the impurities profiles and visualize results, hierarchical clustering analysis was performed using Matlab®. The comparisons were made using a modified Pearson coefficient and the linkage method of unweighted average distance (also known as group average); the output is a dendogram.

Results: In about half of the investigated cases, the physical characteristics links were confirmed with organic impurities analyses. In the remaining cases, the physical characteristics classes were divided in several organic impurities sub-groups, thus supporting the hypothesis that several production batches of MDMA powder were used to produce one single tabletting batch (*i.e.* tablets having the same shape, diameter, thickness, weight and score; but different organic impurities composition). Physical features (except logo and colour) were observed to be quite persistent in time. They provided means to link tablets produced over a long period of time by tabletting machines with given settings. Physical links may therefore be very powerful intelligence tools, especially for investigation purposes, while the added value and combination of chemical links with physical characteristics may be more adequate to refine the investigators' view and to reach court requirements. Organic impurities were demonstrated to be more variable and to have a shorter lifetime than physical features. For this reason, organic impurities have a higher evidential value.

Conclusions: Both sets of characteristics were found relevant alone and combined together. They actually provided complementary information about MDMA illicit production and trafficking. The obtained results will be presented and discussed through illustrating examples.

Keywords: drug profiling, organic impurities, physical characteristics, GC-MS, chemometrics

Free topics

P154. Can we use serum leptin concentration to detect smoking cannabis?

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Introduction: Leptin is an adipocyte-derived hormone that is secreted in correlation with total body lipid stores. Serum leptin levels are lowered by the loss of body fat mass that would accompany starvation and malnutrition. Recently, leptin has been shown to modulate innate immune responses such as macrophage phagocytosis and cytokine synthesis in vitro. Cannabis is one of the world's oldest cultivated plants. Currently, however cannabis cultivation and use is illegal or legally restricted around the globe. Despite constant official control, cannabis cultivation and use has spread to every continent and nearly every nation. Cannabinoid components of cannabis are known to exert behavioral and psychotropic effects but also to possess therapeutic properties including analgesia, cular hypotension, and antiemesis.

Aim: This study aimed to determine the effect of smoking cannabis on leptin concentration, comparing with healthy subjects.

Methods: (GII)10 male with 9-carboxy-tetrahydrocanabinol (THC) was detected in their urine by qualitative analysis by Gas Chromatography-Mass spectrometry (Agilent 6080N-capillary coloumn 30 m-1 µ diameter) collected from forensic medicin institute chemistry lab. Cairo Egypt, comparing with (GI) 10 control healthy male as volunteers with negative 9-carboxy-tetrahydrocanabinol (THC). Age and body mass index (BMI) were matched, diabetic subjects were excluded,serum leptin concentration was measured by quantitative radio immunoassay (RIA) using a kit supplied from Diagnostic Systems Laboratories (DSL) Inc. (445 medical center BLVB. WEBSTEER TX 77598 USA.The method depend on a non-competitve assay in which the analyte to be measured is "sandwiched" between two antibodies.

Results: There was an increase in mean serum leptin concentrations in (GI) 6.47 ng/ml (±SD 1.39), than that in mean in serum leptin concentrations (GII) 4.7 ng/ml (±SD 0.84).

Conclusion: serum leptin concentration may be use to detect smoking cannabis.
Keywords: leptin, THC, cannabis, RIA

P155. Origin of acetaldehyde after ethanol dosing

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Introduction: Acetaldehyde (AcH), the first metabolite of ethanol (EtOH), induces some un-comfortable situation in some Asian populations due to the deficiency of ALDH2 enzyme activity either in part or wholly. Blood AcH is originated mainly by hepatic metabolism of EtOH. Based on several in vitro animal experiments, it is assumed that both blood AcH and brain catalase are strongly involved in the accumulation of AcH in the brain.
Aim: The purpose of our work is to confirm the AcH formation after EtOH dosing by in vivo animal experiments.
Methods: Male Wister rats and mice (ALDH2 knockout (KO) and wild-type) were used in this study. Rats were treated intraperitoneally (i.p.) with EtOH (1 g/kg) alone or in combination with 4-methylpyrazole (4-MP)-ADH inhibitor, cyanamide-ALDH inhibitor, and sodium azide (AZ) or 3-amino-1,2,4-triazole(AT)-catalase inhibitor. Mice were treated with EtOH (2 and 4 g/kg, i.p.) alone. The dialysate samples were collected in the brain by microdialysis and then EtOH and AcH concentrations were determined by head-space GC.
Results: In rats and wild mice dosed with EtOH alone, blood and brain levels of EtOH were almost same. A little AcH was detected in the blood while no detection of brain AcH was found. In rats pretreated with CY, high levels of AcH were produced in the blood after EtOH dosing, which were about five-fold higher than those in the brain. Brain levels of AcH after EtOH treatment were significantly lower in rats treated with AZ or AT than CY. This reduction of brain AcH is due probably to the inhibition of catalase activity by AT or AZ. No detection of AcH in the blood and brain was observed in rats treated with EtOH combined with 4-MP. In ALDH2KO mice, high levels of AcH were produced in the blood after EtOH dosing, which were significantly higher than those in the brain.
Conclusion: Our observation suggests that the hepatic metabolism of EtOH produced AcH in the blood, and catalase, on the other hand, produced AcH in the brain. However, additional experiments will be needed to confirm AcH production through catalase in the brain of ALDH2-deficient mice models.
Keywords: acetaldehyde, ethanol dosing

P156. New aspects of biocrime: skunks anal sac defensive spray

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Introduction: Our laboratory has been solicitated by judicial authorities to elucidate the origin a strong "toxic" smell present in a politician office, private house and motorcar. This stinky and pungent atmosphere has caused serious nausea and vomiting to several individuals. The politician has immediately suspected a "chemical attack" against her office and against her person to be at the origin of this incident.
Aim: The purpose of the work was to identify the compounds responsible for the repugnant smell.
Methods: Drops and swabs of an oily liquid found at the implicated places were submitted by police to our laboratory. Urine samples were collected from the persons presenting symptoms of nausea for toxicological analysis. All analysis were performed using GC/MS and HS-GC/MS.
Results: HS-GC-MS and GC/MS revealed the presence of several compounds which were identified by detailed spectra interpretation as they were not included in any commercial MS-library. (E)-2-butene-1-thiol, S-(E)-2-butenyl thioacetate, 3-methyl-1-butanethiol, S-3-methylbutanyl thioacetate and carbon disulfide (CS_2) were identified using HS-GC/MS. 2-methylquinoline or isomer, 4-methylquinoline, 2-quinolinemethane-thiol, S-2-quinolinemethyl thioacetate, 2-phenylethanethiol, bis (E)-2-butenyl disulphide and bis (3-methylbutyl) disulphide were identified by GC/MS. Some minor peaks present in the chromatogram could not be identified. No other common toxic substances have been detected. A literature research revealed that these identified compounds are present in the defence spray of skunks. In the patients urine it was not possible to detect any volatile substances except trace amounts of ethanol and acetone. Comprehensive drug screening was negative. The repugnant smell lasted for several days. During this period nobody could enter the office and home of the politician. Some of the identified compounds are commercially available and may be used in "chemical attacks" to generate psychological threat and stress due to the repugnant smell, but are otherwise harmless.
Conclusion: Using GC/MS and HS-GC/FID techniques we were able to identify the substances implicated in the "biochemical" attack. Fortunately, no sequels were observed for any of the persons implicated in this case, but as far as we know the offender(s) have never been identified.
Keywords: biochemical attack, skunk defensive spray, MS identification

P157. Demonstration of pyrolysis products in blood from a charred cadaver by cryogenic gas chromatography-mass spectrometry following low-temperature headspace solid-phase microextraction

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Introduction : In the determination of death by fire, vital signs, such as burn, aspiration of soot, carbon monoxide and other combustion gas or ignitable liquid components are very important. It is also very important to analyze volatile hydrocarbons in the blood of the charred body, especially when the vital signs of the charred cadaver are poor. Therefore, examination of volatile hydrocarbons in blood in addition to general drug screening test plays an important role in forensic investigation.
Aim: An autopsy was performed on a charred male found in a forest. Forensic pathological findings included only poor vital signs. The blood carbon monoxide hemoglobin saturate was less than 10%. Although some toxicological analyses were performed by gas chromatography-mass spectrometry (GC-MS) and GC, there were no routine drugs detected. Then, to find some evidence indicating death by fire, the heart blood was analyzed by GC-MS following headspace solid-phase micro-extraction. The utility of this volatile analysis was investigated.
Methods: Blood was obtained at autopsy. Then 0.1 ml of blood was placed in a headspace vial containing 1 ml of fresh tap water without petrol components and the solution was warmed at 80 °C for 10 min, and then left standing for 2 min at room temperature. The solid phase microextraction (SPME) fiber, 65 μm PDMS/PVB, was inserted in the vial through a silicone-rubber seal and the analytes were absorbed on the fiber at 0 °C for 15 min. The analytes were desorbed at 250 °C in a liner for GC-MS analysis. A Shimadzu QP-2010Plus was used under cooling initially at -40 °C. The column used in this study was an Rtx-5, 10 m × 0.18 mm i.d., 0.2 μm of film thickness.
Results: Benzene, toluene, phenylethyne, styrene, indene, naphthalene and so on were detected, but the typical petrol pattern indicating octane, dodecane, n-propylbenzene, trimethylbenzene and other components was poorly observed. Indene and naphthalene may be generated from pyrolysis products being decomposed at high temperature under a low oxygen environment.

Conclusion: The detection of these compounds, phenylethyne, indene and naphthalene, supports the vital signs indicating that the body inhaled high temperature air in the ignition.
Keywords: pyrolysis products, blood, cadaver

P158. UFLC-MS/MS as screening and/or quantification tool for analysis of 28 benzodiazepines and non-benzodiazepine related hypnotics in urine and whole blood

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Introduction: Benzodiazepines and non-benzodiazepine related hypnotics are widely (ab)used in Belgium. Therefore, efficient analytical methods are needed for screening and quantification of these compounds. The application of LC using small particle sizes with tandem mass spectrometry will offer the needed sensitivity and selectivity.
Aim: The purpose was to develop a UFLC-MS/MS method for the analysis of 25 benzodiazepines, its metabolites and 3 non-benzodiazepine related hypnotics that are (ab)used in Belgium: alprazolam, 7-aminoflunitrazepam, bromazepam, brotizolam, chlornordazepam, clonazepam, clobazam, clotiazepam, desmethylflunitrazepam, diazepam, ethylloflazepate, flunitrazepam, flurazepam, lorazepam, lormetazepam, midazolam, nitrazepam, nordazepam, oxazepam, prazepam, temazepam, tetrazepam, triazolam, zaleplone, zolpidem and zopiclone.
Methods: For screening purposes, the urine was simply diluted and injected onto the LC-MS/MS (Shimadzu Prominence UFLC coupled to 3200 QTRAP, Applied Biosystems). For confirmation, an efficient SPE procedure using mixed-mode Bond Elut Certify columns was applied to urine and whole blood samples. The analytes were separated on a ShimPack XR C18 column (2.2 µm, 3.0 × 75 mm) with gradient elution using aqueous ammonium acetate and methanol. For each analyte, two MRM transitions were monitored. APCI in positive mode was selected as ionization mode. N-methyl-clonazepam was used as internal standard.
Results: A comparison between two ionization sources, APCI and ESI, showed that APCI was most efficient for most compounds. In 7 minutes, all 25 benzodiazepines, metabolites and 3 non-benzodiazepine related hypnotics could be detected. The method was fully validated: selectivity, precision, linearity, LOD, LOQ, precision, recovery and matrix effects were evaluated. The method was then successfully used for the analysis of urine and whole blood in the context of forensic cases.
Conclusion: To the best of our knowledge, this is the first LC-MS/MS method using a column with small particle size to analyze 28 hypnotics. The sensitive and selective UFLC-MS/MS method was validated and successfully applied to forensic cases for analysis of 25 benzodiazepines, its metabolites and 3 non-benzodiazepine related hypnotics.
Keywords: UFLC, MS/MS, urine, blood, benzodiazepine

P159. Development of a guideline for alcohol analysis in forensic specimens by gas chromatography-headspace in Thailand

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Introduction: The Forensic Toxicology Network of Thailand (FTNT) has been founded in March 2008. Now, there are 106 members from 3 Institutes, 7 Universities, 8 Regional Medical Science Centers, 5 local hospitals and 1 private lab that analyse forensic specimens. The first goal of our FTNT is to develop an internationally acceptable standard of practice. The guideline for alcohol analysis in forensic specimens by GC-HS was drafted and proposed firstly to the members of FTNT and TIAFT's executive committee during March 2009 at the Central Institute of Forensic Science, Bangkok.
Aims: To develop the national guideline for alcohol analysis in forensic specimens by GC-HS in Thailand.
Methods: A questionnaire was firstly drafted and sent to 17 toxicology laboratories prior to modification of the original guideline or SOP of alcohol analysis of the Regional Medical Sciences Center, Chonburi, Thailand. Information from international toxicology laboratories was reviewed and compaired to the obtained data from Thai laboratories. All the data were presented and discussed between attending FTNT members and TIAFT's executive committee in order to reach an unanimous conclusion.
Results: Table shows the data from Thai, International and SOFT guideline. The numbers indicate the number of laboratories that currently comply with the recommended features.

Characteristic	Thailand n (%)	International n (%)	SOFT Guideline
Calibrator (levels) - 3 levels (at least)	11(92%)	6 (100%)	3 levels
QC level - 1 level (at least)	12 (100%)	6 (100%)	1 level
Sequence of injection sample and QC - each batch of samples include at least 10% of control	10 (83%)	5 (83%)	each batch of samples includes at least 10% controls
No. of injections/sample - 2 (at least)	11(92%)	6 (100%)	2
Technique – GC-HS	12 (100%)	6 (100%)	GC-HS
Reporting unit - mg%, g%	12 (100%)	6 (100%)	mg%, g%
QC acceptance - ±5% (at least)	7 (58)%	2 (33%)	20%
ISO, International accredited	5 (42%)	6 (100%)	-

This is the first guideline of FTNT. Details of the guideline were written in Thai and will be sent to all members of FTNT in April 2009.
Conclusions: The national guideline for alcohol analysis in forensic specimens by GC-HS in Thailand has been developed and it meets the minimum requirements of an international standard. Members of FTNT will receive the guideline and the recommendation to use it in their lab. The guideline will be a "living document" and it will be updated at when appropriate.
Keywords: guideline, alcohol analysis, gas chromatography

P160. Systematic toxicological screening analysis using ultra performance liquid chromatography with ultra violet and mass spectrometry detection (UPLC-UV/MS)

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Introduction: Systematic toxicological screening analysis (STA) is used to highlight the presence or the absence of a panel of xenobiotics in an unknown sample. Three chromatography based techniques are mainly used for STA: HPLC/UV, GC/MS and LC/MS. In the field of liquid chromatography, Ultra Performance LC® (UPLC) brings interesting benefits to analytical chemistry with reduced analysis time, enhanced resolution and sensitivity, while using a reduce solvent consumption. If most of the STA techniques developed with UPLC used MS detection because of its high specificity, UV detection still interesting when semi-quantitative results are required.
Aim: The aim of this work was to develop a new STA procedure with semi-quantitative capabilities by UPLC/UV/MS.

Methods: Biological samples (blood, urine, plasma, gastric content) were analysed by using a liquid/liquid extraction (Toxitube A®). The chromatographic system used was an Acquity™ UPLC/PDA/TQD (Waters, Saint Quentin en Yvelines, France). LC conditions were as follow: column: Acquity™ UPLC BEH C18 (150 mm × 2.1 mm, 1.7 µm); column temperature: 50 °C; gradient composition and profile: solvent A: Acetonitrile/5 mM Ammonium Acetate buffer pH 3.8 (15/85 – v/v), solvent B: Acetonitrile; profile: 100% A for 1.4 min then 89% A in 1.4 min (linear) then 75% B in 8.2 min (linear) then stay at 75% B for 0.2 min before going back to initial conditions; flow rate was constant at 450 µL/min. A large panel of compounds was used to build two specific databases: an UV library and an MS database. Performances of the method have been evaluated by comparison with a previously published HPLC/UV method routinely used in our laboratory.

Results: The method provides a short runtime analysis (injection each 15 min) with a good resolution and a good peak capacity. More than 600 compounds were registered with both UV and MS data. Some metabolites, such as 3-hydroxy-bromazepam, 9-hydroxy-risperidone or cyamemazine sulfoxide, were identified and included in the databases. The UPLC/UV/MS is faster and provides more resolution compared to the reference HPLC/UV technique routinely used in our laboratory.

Conclusions: The UPLC/UV/MS procedure proposed is fast, resolutive and rug. Compounds identification is based on RT, UV spectra and m/z ratio by comparison with 2 libraries containing about 700 entries. This method combines the specificity of the MS detection and the quantitative interest of the UV detection. This STA procedure is routinely used either for clinical or forensic toxicology screening.

Keywords: systematic toxicological analysis, UPLC/UV/MS, biological samples

P161. Analysis and reported health effects of illegal weight-loss pills

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Introduction: With the ideal of beauty being slim, and the increasing problem of overweight in Western society, there is an increasing use of weight-loss preparations. Concomitantly, the (internet) trade in illegal or counterfeit weight-loss medicines and dietary supplements, intended for losing weight, is growing.

Aim: To investigate the composition of suspect weight-loss preparations and to identify possible health risks related to the use of these products.

Methods: Suspect weight-loss medicines and dietary supplements were confiscated outside of the official distribution chain by several governmental and public institutions involved in law enforcement. The motive to perform analysis was a suspected violation of the Commodities Act, Medicines Act or Tax and Trade laws. The samples were analyzed in four different laboratories. The analytical results of the samples confiscated between 2002-2007 were compiled by the National Institute of Health and the Environment (RIVM). Furthermore the records of the Dutch Poisons Information Center (DPIC) were searched for reports of adverse health effects associated with the use of weight-loss products in the period 2000-2007.

Results: 256 samples were analyzed of which 44 (17%) were suspect illegal weight-loss medicines and 212 (83%) were suspect dietary supplements. Of the 44 suspect illegal weight-loss medicines 73% were counterfeit medicine and 27% were genuine medicine with a strong increase of the percentage counterfeit medicine after 2005 (counterfeit 90%, genuine 10%). The counterfeit medicines contained either a lower amount of the active ingredient, the wrong active ingredient or no active ingredient at all. The results of the suspect dietary supplements were divided into two time periods 2002-2004 and 2005-2007, because of the prohibition of ephedra-alkaloids in The Netherlands in May 2004. In 2002-2004 122 samples were included of which 88% contained ephedra-alkaloids. Of the 90 samples collected between 2005-2007, after the ban on ephedra, 62% contained ephedra-alkaloids. Another finding was that after 2004 the percentage of dietary supplements adulterated with sibutramine went up from 2% to 23%. In the DPIC records from 2000-2004, there was a rise in the number of enquiries about ephedra (from <30 to 127) and a decline to the old level (<30) after the ban on ephedra in 2004.

Conclusion: Weight-loss medicines that are not obtained via the official distribution chain and dietary supplements for weight-loss mostly contain ephedrine-alkaloids or sibutramine in moderate to high dosage. The ban on ephedra did not result in the disappearance of ephedra-alkaloids from dietary supplements for weight-loss. Compared with the analytical results, the decline in the number of enquiries concerning ephedra to the DPIC is greater than expected. This is probably due to the fact that ephedra is not declared on the label anymore, thus posing an additional health risk to the unwitting user.

Keywords: counterfeit weight-loss medicines, dietary supplements, sibutramine, ephedrine-alkaloids

P162. Evaluation of a GC/MS drugs of abuse confirmation kit based around new GC/MS software

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Introduction: When a new laboratory wants to add the ability to do drugs of abuse testing, methodology has to be created and validated before real samples can be tested. This process can be arduous and time consuming, particularly if many of the lab personnel are new to toxicology analysis. Presented is a description of the evaluation of a kit which allows for fast start up for common GC/MS confirmation assays. Also presented is an evaluation of a new GC/MS software package which is included in the kit and is intended to streamline method development, batch setup, data acquisition and review, and report generation.

Aim: To evaluate the utility of the kit to accelerate method setup, and to test the methods contained in the kit for linearity, precision and specificity performance.

Methods: In addition to the new GC/MS operating software, the kit includes preloaded validated methods for the hydrolysis, extraction, derivatization and single quadrupole GC/MS analysis of carboxy-THC, PCP, BE, amphetamines and opiates in a urine matrix. Also contained in the kit are many of the items needed for method re-validation and daily operation of the instrument. These items include necessary consumables, a quick start guide, and standard operating procedures. The materials and methods contained in the kit where implemented on a new GC/MS to test its performance for method linearity, precision and specificity of the five drug classes.

Results: The following is example data from linearity and inter-day precision studies for the methods contained in the kit:

Drug	LOD (ng/mL)	LOQ (ng/mL)	ULOL (ng/mL)	Precision at Cutoff	Precison @ 40% QC	Precision @ 125% QC
THCA	1.5	1.5	1,000	2.9%	1.3%	4.3%
BE	15	15	12,500	0.7%	1.1%	2.3%
PCP	5	5	5,000	1.6%	1.1%	1.9%
AM	25	25	50,000	1.6%	1.9%	1.3%
AM (200 ng/mL)				2.2%	1.2%	2.1%
MA	25	25	25,000	4.3%	4.6%	4.4%
Codeine	60	60	50,000	1.4%	1.4%	3.3%
Morphine	100	100	50,000	1.8%	1.0%	5.5%

Conclusion: The methodology and contents in the kit proved to be a useful tool to quickly validate these assays on a new instrument and gave excellent quantitative performance during the validation process.
Keywords: GC/MS, drugs of abuse, software

P163. Assessment of human inhalation exposure to lead due to candles wicks's emission. The Greek experience

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Introduction: Despite the fact that the use of lead in wick cores has been forbidden in many countries among them U.S. since 1974, many of the candles sold in Greece even today contain various amounts of lead in their cores. Inhalation exposure to lead may cause a number of hematopoietic problems that are related to human health as well as many neurological (cognitive) deficits in young children.

Aims: The aims of this study were the following: 1) the determination of the exact quantity of lead per centimeter of candle cores, that exist in candle, sold in the Greek market, 2) the extrapolation of the burned lead to concentrations in indoor air pollution and 3) the correlation of exposure rates with possible blood-lead-levels and the specific neurological side-effects in children.

Results and Conclusions: One hundred and ten different kinds of candles lead examined for the needs of this study and 31.8% of them where found to contain wick cores. The lead content of these wicks was found to be between 13.4 mg/cm and 39.7 mg/cm of lead wick. These findings are discussed in detail in relation to the effects of lead in human health.

Keywords: candles, lead, inhalation exposure

P164. The protective effects of melatonin and zinc in acute cadmium nephrotoxicity: toxicological, histological and ultrastructural study

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Introduction: Cadmium (Cd) is an important occupational and environmental pollutant. Cd is produced and refined as a by product of other metals such as zinc and lead and it is extensively used in planting, alloys, catalysts, pigments, plastic stabilizers and batteries. Cd- contaminated soil and water may occur naturally or as a result of emissions from industrials, the use of Cd-rich fertilizers and /or sewage (sludge), or in the form of deposited air pollution.

Aim: This study was performed to assess the acute hazardous effects of cadmium on the kidney and to assess the protective role of both melatonin and zinc.

Method: Forty adult male albino rats were divided into four groups: control, cadmium, cadmium and melatonin and cadmium and zinc groups. Kidney functions, histological and ultrastrucural studies were done.

Results: The blood urea, uric acid and creatinine values showed deterioration in the cadmium group but were improved in cadmium and melatonin than in cadmium and zinc groups. In the cadmium group the histological results showed glomerular congestion, atrophy, interstitial haemorrhage and degeneration of the proximal convoluted tubules. These findings were improved in the cadmium and melatonin group than in the cadmium and zinc group which was partially improved. Morphometric measurements showed that melatonin protected the kidney more than zinc from cadmium toxicity as regards the optical density of basement membrane of renal corpuscle, glomerular area and proximal convoluted tubule epithelial height. In cadmium group the ultrastructural results showed cellular degeneration of the proximal tubules in the form of vacuolated cytoplasm, mitochondrial degeneration and irregular nuclear membrane. The glomeruli showed marked thickening of the basement membrane. In cadmium and Melatonin group there was excess lysosomes only. In cadmium and zinc group the nuclei were pyknotic, cytoplasm vacuolated and glomeruli showed thickening of the basement membrane.

Conclusion: It is concluded from the present work that melatonin protected the kidney from cadmium more than zinc as detected from histological and morphometric results and obviously seen on the ultrastructural level.

P165. Simultaneous extraction of different organic phosphorus pesticides from biological samples by solid phase extraction and determination by gas chromatography coupled with pulsed flame photometric detector

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Introduction: Judging from the application of pesticide, organic phosphorus pesticide had the biggest application amount, followed by organic nitrogen and trifluorocypermethrin pesticides. Organic phosphorus pesticides constitute a very important group owing to their extended using in agricultural practice and many kinds of crimes, such as suicide.

Aim: The purpose of our work was to develop a simple and efficient analytical method for simultaneous determination of different species of organic phosphorus pesticides from biological samples by using solid phase extraction.

Methods: Firstly, the developed extraction method was validated using certified reference materials of biological organic and applied to biological samples. Secondly, the developed extraction method was validated by implementing gas chromatography coupled with pulsed flame photometric detector as a diagnostic tool.

Results: This extraction method for multispecies analysis incorporates many benefits in terms of speed, low solvent use, accuracy of measurement, sensitivity, relative simplicity, as well as the time saving and convenience of multiple species measurement through sample preparation and analysis as an integrated step.

Conclusion: A simultaneous extraction and determination method with selectivity and sensitivity required for organic phosphorus pesticides was obtained through the optimized extraction step and the GC/PFPD parameters.

Keywords: organic phosphorus pesticide, GC/PFPD, biological sample, SPE

P166. Special testing and 3D optical measurements in cases of supposable counterfeit medications

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Introduction: The description of seized illicit or counterfeit tablets and other pressed drug products is an important step in casework. The physical and visual analysis and the description of the characteristics can be employed for intelligence purposes. In this work the method of 3D surface digitizing is introduced as a suitable tool for high accurate documentation of small objects, especially for pressed drug products. The resulting detailed information about the geometry and the results of an automatic comparison of apparent uniform tablets allows distinguishing genuine and counterfeit medications.

Methods: Original and counterfeit Viagra® tablets were digitized by the use of the GOM ATOS II system (GOM, Braunschweig, Germany [1]).

A projector unit projected a fringe pattern onto the surface of the object, which was recorded by two CCD cameras mounted besides the projector. The sensor unit was connected to a High End PC. All captured images were instantly transferred. Based on the principle of triangulation, 3D coordinates of up to 1.3 million surface points per measurement were calculated by the scanning software ATOS. The ATOS II SO setup with objectives of 50 mm focal length (projector and cameras) was calibrated for a measuring volume of 35 × 28 × 15 mm, with a resulting point spacing of 0.03 mm - The measuring noise amounted to 0.001 mm. The tablets were fixed in a 28 × 28 mm frame. Adhesive circular markers were applied to the frame. These markers served as reference targets for merging the single measurements, done from different views, automatically into a complete 3D data set [2].

Results: The results of the software aided measurement of the dimensions of the analyzed tablets displayed negligible variations. The method allows distinguishing counterfeit and genuine medications. Further analysis *e.g.* impurity profiling and quantitative determination of the pharmacological active ingredients with GC-MS and HPLC-DAD confirm that counterfeit medications are usually not safe.

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P167. A comparison of toxicological analysis reports of two statutory laboratories: the need for accreditation and education

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Introduction: In India, today we have about 23 full fledged forensic science laboratories both under the centre and the states. In some states, the chemical examiner's laboratory is merged with the forensic laboratory, whereas in a few states, they are functioning independently. Based on the scientific reports from government institutions like forensic science laboratories, chemical examiner's laboratory, various courts in our country admit the reports under section 45 of the Indian Evidence Act. Under section 293 Criminal Procedure Code of India the report of certain government scientific experts may be used as evidence in any inquiry, trial or other proceeding under this Code, as such. Acknowledging the need to speed up the analysis of cases related to viscera examination at laboratories the Directorate of Forensic Science, Ministry of Home Affairs, Government of India has constituted an important committee to look into the problem areas of toxicological analysis.

Aims: To compare the quality of reports issued from two different government laboratories and review the conclusion in view of the facilities available in the lab and the wording of the opinions and the implications thereof.

Methods: In most parts of India, in cases of medico-legal autopsy where poisoning is suspected, the final opinion as to the cause of death is generally kept reserved, pending the chemical analysis report from the forensic science laboratory or the chemical examiner laboratory. These chemical laboratories are usually the only one of its kind to examine human viscera sent after *post mortem* in cases of murder and unnatural deaths, semen examination in cases of rape and cases of poisoning in attempted homicide and suicide. The laboratories also examine samples of opium, alcohol and other drugs under the Prevention of Narcotics Act. The reports received from two of these state laboratories in *post mortem* cases have been compared for the content, concluding remarks and knowledge of the process.

Results: One of the labs is accredited with the chemists and scientists being properly qualified and educated about the process. The other lab is being run by medical doctors who are neither trained nor qualified for conducting these examinations; neither do they have the necessary facilities for carrying out these tests. How the reports are issued is anyones guess and though the issue is raised periodically in the media but nothing concrete comes out. That these reports have to be used in the court of law as evidence and most of the times even without cross examination, highlights the role that these reports have in meting out justice.

Conclusions: The report of the chemical examiner must contain reasons for arriving at a particular conclusion, disclose the tests of experiments performed by the chemical examiner, the factual data revealed by such tests or experiments and the reasons which led the chemical examiner to form his opinion, so as to enable the Court to arrive at its own independent conclusion. The report must contain all the information which the chemical examiner would have furnished, had he been called and examined as a witness in the case. Where the matter to be reported on is the presence of certain substance in the article submitted for examination, much would turn on the quantity of the incriminating substance found in the article and that must be mentioned in the report. When the report is meagre or cryptic, the Court should call for a detailed report or examine the chemical examiner. All these points highlight the importance of the need for accreditation of these laboratories regularly and the need for educating the concerned officials about the necessity of the same.

P168. The protective effect of silymarin on the suprarenal gland in chlorpyrifos intoxicated rats

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Introduction: Chlorpyrifos (CPF) is a widely used organophosphorous insecticide that induces toxic effects in man and animal through its inhibitory action on acetyl choline esterase enzyme.

Aim: The present work aimed to evaluate the toxic effect of chlorpyrifos on the function and structure of the suprarenal gland and to investigate the possible protective effect of silymarin antioxidant against such toxicity.

Methods: The study included twenty four adult male rats they were equally divided into four groups as follows: a control group (n=6) received the oral vehicle only (corn oil), a Silymarin group (n=6) received silymarin in a dose of 6 mg/kg b.w orally twice weekly for four weeks, the intoxicated group (n=6) received CPF 5 mg/kg b.w. orally twice weekly for four weeks and the protection group (n=6) received silymarin orally 6 mg/kg b.w. half an hour after chlorpyrifos administration in a dose of 5 mg/kg b.w. orally twice weekly for four weeks. By the end of the experiment, estimation of the following biochemical parameters was done: plasma choline esterase enzyme activity level, serum cholesterol serum cortisol and serum testosterone. The suprarenal gland was examined by the light microscope using routine H&E stain and chromaffin stain reaction, as well as by the transmission electron microscope.

Results: The measured biochemical parameters showed significant variation in CPF group compared to the control. The chlorpyrifos intoxicated group revealed affection of the cells of the suprarenal cortex and medulla with variable degrees of degenerative changes. The protection group showed improvement of the levels of the biochemical parameters with partial restoration of the normal histological features of the suprarenal structure compared to the CPF intoxicated group.

Conclusion: It was proved that silymarin is a reliable antioxidant that could protect against the toxic effect of chlorpyrifos on the suprarenal gland.

Keywords: chlorpyrifos, silymarin, suprarenal gland

P169. Pesticide intoxication problems in Cukurova Region, Turkey: a 3 years analysis

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Introduction: Cukurova region is the most important agricultural area for Turkey. As a consequence of wide pesticide use, acute pesticide poisoning cases are quite common serious problem in this region. These poisonings are generally suicidal self poisonings, while some can be accidental or homicidal as well. In Cukurova region, pesticide poisoning still remains as a significant cause of death, which led the present study.

Aims: The autopsy records of Adana Group Authority of the Council of Forensic Medicine, between 2006 and 2008, were evaluated retrospectively. Deaths that are attributed to pesticide poisoning included in the scope of this study in order to identify the type of the pesticide, as well as the etiology. The frequency and distribution of intoxications were also analyzed in terms of sex and age.

Methods: In the study period, total of 4199 autopsies had been referred to the forensic toxicology laboratory for pesticide analysis. Pesticide analyses were performed in the Forensic Toxicology Laboratory of Adana Group Authority of the Council of Forensic Medicine.

Results: Seventy-two out of all cases were positive for pesticide analysis. Of these 72 cases, 42 (58.33%) were male and 30 (41.66%) were female, with a mean age of 38.8 ± 20.6 years. Among the pesticides inspected, endosulfan was found to be the most common with 47.2% prevalence· followed by the organophosphorus insecticide dichlorvos with 16.7% prevalence. Majority of deaths due to pesticide poisonings (37, 51.38%) were suicidal while 17 (23.61%) of them were accidental.

Conclusions: This report showed that endosulfan, an organochlorine pesticide, is commonly used in Cukurova region. Moreover, frequency of acute and chronic exposure to endosulfan is considerably high in Cukurova region. Recently, strict regulations have been been enacted for restricting and controlling the use of endosulfan, which previously allowed to use. Furthermore, authorities should set up more efficient educational facilities for agricultural workers in order to reduce the number of accidental pesticide poisonings.

Keywords: forensic toxicology, pesticide poisoning, autopsy, cukurova

P170. Exposure to lead – a case study of female bangle makers

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Introduction: Indian women, traditionally wear bangle. Bangles manufactured at various places in India. In bangle making industries workers working nearest high pot furnace that may have a temperature up to 1600 degree Celsius. Workers inhale soot, fumes and dust from the dry glass mixture and other chemicals. Work environment offer sufficient chances of exposure to lead. Toxicity of lead has been associated with imbalance in heme synthesis and in enzyme activity. Various manifestations of lead toxicity have been studied through epidemiologic investigations (WHO, 1995).

Aim: The aim of this study was to observe possibility of exposure to lead in female population engaged in bangle making industries. Subjects include women of various age groups.

Methods: Forty female subjects of various age groups recruited for this study. A questionnaire was prepared to collect information on their age, duration of exposure, smoking habit, alcohol intake and food habit. Urine samples from these workers were collected after work shift. Specific gravity recorded using urinometer. Creatinine was determined through a commercial kit (Toro and Ackerman, 1975). Lead determined by atomic absorption spectrophotometrically (AAS). Delta–ALA determined by the method of Tomokuni and Ogata (1972).

Result: We have observed that lead toxicity varied amongst female workers of different ages. Variations in the relationship between urinary lead concentrations and ALAU values were also observed. ALAU was found to be higher in smoker than nonsmokers. Lead values in urine samples were found higher than healthy subjects.

Conclusion: Our results indicated that there is exposure of lead among females engaged in industries. It is possible that individuals with certain ALAD polymorphism may be more susceptible to the effect of Lead.

Keywords: lead, delta ALA, occupational exposure, bangle makers

P171. The determination of Δ^9-THC metabolites in meconium: a pilot study in Cyprus

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Introduction: Identification of intrauterine drug-exposed newborns may have benefits including close follow-up of the infant by both medical and social services. Neonates that are exposed to cannabinoids in utero may have characteristic physical and mental developmental problems throughout their lives. It is therefore desirable to diagnose and begin treatment of these individuals as early as possible.

Although urine and blood are the most obvious specimens to collect for detection of prenatal exposure to drugs, they only provide information about recent drug exposure (two or three days before birth). Meconium is an ideal specimen for analysis of drugs and metabolites in the newborn period for the following reasons: (a) the collection of meconium is simple and noninvasive, (b) meconium is available for up to three days after delivery, (c) initial testing can be performed with common laboratory techniques for screening, and confirmatory testing using gas chromatography mass spectrometry (GC/MS), (d) testing is sensitive and specific, (e) it is a window to the last 30 weeks of gestation. The disadvantage to meconium testing is the increased labor and the extra time needed for the clean up procedure. According to the statistical information, cannabis is today the most widely abused illicit drug in the world. Cannabis has been used for its euphoric effects for over 4000 years, Δ9-tetrahydrocannabinol (THC), the primary psychoactive analyte, is found in the flowery or fruity tops, leaves and resin of the plant. THC with cannabidiol (CBD) and cannabinol (CBN) are the tree main constituents presently isolated from the *Cannabis sativa* plant. THC is extensively metabolized in two main metabolites: 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol (THC-COOH) and 11-hydroxy-9-Δ-9-tetrahydrocannabinol (11-OH-THC) are detectable in biological matrices. Only the hydroxy-metabolite has a psychoactive effect. The major THC metabolites found in meconium may be different from those in urine and blood. THC and its 8-hydroxy-9-Δ-9-tetrahydrocannabinol (8-OH-THC) are either not detected or present in low concentrations in meconium specimens screening positive for cannabinoids.

Aim of the Study: The aim of this study was to investigate the prevalence of cannabis use during pregnancy in Cyprus and target social groups or age groups for training and information prevention purposes.

Experimental: Samples of meconium were collected from neonates born in two main districts in Cyprus, during one year period. Approximately 200 samples were collected, with the indication for Cypriot or Foreign neonates. Screening was performed using ELISA and confirmation was performed by GC-MS and LC-MS-MS.

Results: Cannabis metabolites were detected in a small percentage of samples mainly from foreign mothers.

Conclusions: This pilot study will be extended also in other Cyprus districts as well as in other kinds of drugs of abuse because the use of narcotics poses a serious problem to newborns since mental and physical problems may arise during their development. They are the youngest victims of substance abuse and prevention policies and actions must be supported by scientific data.

INDEX

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z