ORAL PRESENTATIONS

SFTA Meeting

1. Prise en charge d'un état d'agitation aux Urgences

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Introduction : Les problèmes posés par la prise en charge d'un patient agité dans un service d'Urgences sont multiples : médicaux, médico-légaux et organisationnels. La présence d'un seul patient agité peut compromettre le fonctionnement de tout un service! La meilleure réponse réside dans une organisation très structurée de la prise en charge de ces patients.

Points clé de la prise en charge :

Alerte : Le plus souvent, la prise en charge d'un patient agité a débuté en amont de l'hôpital, idéalement par un appel au SAMU. Il est capital que le service des Urgences soit alerté au plus tôt de l'arrivée du patient pour préparer son accueil. Un circuit spécifique limitant les contacts avec le patient est recommandé.

Accueil : Si les causes psychiatriques (60 %) et toxicologiques (25 %) d'agitation prédominent, elles constituent des diagnostics d'élimination qui justifient, pour de nombreux auteurs, un examen initial par un médecin somaticien. La prise en charge immédiate par l'infirmière d'accueil, le médecin urgentiste et (idéalement) le psychiatre est à privilégier. L'examen clinique est souvent difficile mais infiniment plus rentable que des examens complémentaires tous azimuts. L'objectif premier est d'identifier rapidement les patients relevant d'un traitement spécifique urgent.

Orientation : Si le patient a des antécédents psychiatriques avérés, un examen clinique (incluant les mesures de SpO2 et de glycémie capillaire) normal, il est confié au psychiatre. Dans le cas contraire, les examens sont poursuivis selon les constatations cliniques. Un « screening » toxicologique est intellectuellement et économiquement inadapté et son rendement nul. Au contraire, un bilan toxicologique orienté est utile et rentable.

L'alcool est la principale cause toxique d'agitation. Seul un examen clinique rigoureux permet de réduire le risque de méconnaître une autre affection (qui peut être associée à une intoxication éthylique aiguë). Le dosage d'alcoolémie n'est même pas indispensable si le tableau clinique et l'évolution sont cohérents.

Contention : Elle ne doit pas intervenir avant une tentative de prise en charge relationnelle. Elle est souvent efficace si les conditions de son succès sont réunies : locaux adaptés, personnel formé, disponible en nombre suffisant… La principale question est celle du choix entre contention physique et pharmacologique. La sédation pharmacologique doit être privilégiée. Hors situations psychiatriques, les benzodiazépines sont recommandées, mais en pratique, les neuroleptiques (loxapine) sont plus souvent prescrits. La cyamémazine est préférée dans les situations psychiatriques. La contention physique intervient en plus de la contention pharmacologique. C'est une prescription médicale. Elle obéit à des règles de mise en place très strictes. La surveillance rapprochée du patient sédaté est capitale.

Organisation : Un local adapté et des moyens humains suffisants et spécifiquement formés sont déterminants. Des procédures de prise en charge multidisciplinaire doivent être disponibles et connues de tout le personnel et associées à des formations spécifiques régulières.

Sécurité : Elle est de la responsabilité du directeur de l'établissement.

Conclusion : La prise en charge d'un patient agité dans un service d'Urgence ne peut s'improviser. Multidisciplainaire, elle répond à des nombreuses règles qui ont en commun des mots-clé : organisation et anticipation.

2. Psychose et cannabis, mythe ou réalité ?

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Introduction : La nature du lien entre cannabis et troubles psychotiques n'est pas totalement élucidée. Les personnes souffrant de troubles psychotiques consomment plus fréquemment du cannabis que la population générale. L'hypothèse explicative a longtemps été celle de « l'auto-médication » des symptômes psychotiques par le cannabis. Cette hypothèse a été récemment remise en cause. Le cannabis aggrave la symptomatologie et le pronostic d'une psychose. Par ailleurs, plusieurs études en population générale ont montré que l'exposition au cannabis était associée à un risque accru de développer un trouble psychotique. Une évidence scientifique croissante montre une association robuste et cohérente entre la consommation de cannabis et le développement ultérieur des troubles psychotiques. L'exposition au cannabis pourrait être un facteur de risque pour les troubles psychotiques en interagissant avec une vulnérabilité préexistante pour ces troubles.

Méthodes : une brève présentation des études internationales publiées ces cinq dernières années permettra de mieux appréhender les liens complexes existant entre cannabis et troubles psychotiques, et aidera à répondre à la question sur l'existence de la psychose cannabique aigue.

Résultats : L'hypothèse la plus probable est celle d'une vulnérabilité neurobiologique prédisposant au développement d'une psychose, pour lesquels le cannabis serait un facteur précipitant.

Conclusion : il n'y a actuellement aucune preuve définitive de l'existence d'une relation de cause à effet entre consommation de cannabis et développement de troubles psychotiques, notamment de la schizophrénie. Il semble cependant qu'un usage précoce de cannabis puisse augmenter le risque de développer une psychose, chez des sujets vulnérables.

Mots clés : psychose, cannabis, comorbidité

3. Dosage de l'association chloroquine-proguanil (Savarine®) sur spots de sang séché

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Introduction : Malgré le développement d'important phénomène de résistances, l'association chloroquine-proguanil (Savarine®) est encore très utilisée dans la prophylaxie ou le traitement des accès palustres. Evaluer la fréquence de la résistance, nécessite de connaître exactement la compliance au traitement par des dosages fiables, difficiles à réaliser dans des régions isolées souvent sans infrastructures. Présentant de nombreux avantages, l'utilisation des « spots de sang séché » (dried blood spot, DBS) s'est considérablement développée. Nous rapportons dans ce travail, la validation analytique d'une méthode de dosage de la chloroquine (CQ), du proguanil (PG) et de leurs métabolites sur DBS ainsi que son application chez 10 patients sous prophylaxie avec 1 gélule par jour de Savarine® (100 mg chloroquine/ 200 mg de proguanil).

Méthodes : La préparation des échantillons a comporté une phase d'élution du sang (80 µL) par agitation du spot dans une solution ammoniacale suivi par une extraction solide-liquide sur colonne (C18 BondElut®). La séparation a été réalisée par chromatographie liquide (système Varian®) en gradient d'élution (tampon phosphate 40 mM, pH 5,5 avec variation du pourcentage d'acétonitrile entre 12 et 40 % sur 30 minutes) sur colonne 5 µm X-Terra® (100 x 4,6 mm, i.d.) avec détection à 254 nm, en utilisant un dérivé du cycloguanil (10 mg/L) comme étalon interne. Le temps de rééquilibrage entre 2 échantillons est de 10 min. La gamme et les contrôles ont été préparés sur des spots de papier et traités de façon identique aux échantillons des

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patients. La conservation des spots a été étudiée à quatre températures (- 20 °C, + 4 °C, + 20 °C et + 50 °C) pendant des temps variables (1, 5 et 20 jours). Notre méthode a été validée chez 10 patients sains recevant de la Savarine® à titre prophylactique depuis 6 semaines. Tous les résultats sont exprimés en moyenne ±SEM et ont été comparés statistiquement par un test ANOVA avec $p < 0,05$ comme seuil de significativité.

Résultats : Dans nos conditions, les temps de rétention pour la CQ, la monodeséthylchloroquine (MDCQ), l'étalon interne, le cycloguanil (CG) et le PG sont respectivement de 8,4 ; 10,4 ; 20,2 ; 22,1 et 27,2 min. Notre méthode est linéaire dans la gamme de concentration 150 à 2500 $ng.mL^{-1}$ pour CQ et MDCQ ($R^2 > 0,999$, biais < 7 %) et 300 à 2500 $ng.mL^{-1}$ pour CG et PG ($R^2 > 0,998$, biais < 10 %). Le rendement total d'extraction est compris entre 76 et 91 % selon les molécules. La limite de quantification est de 50 $ng.mL^{-1}$ pour CQ et MDCQ et de 100 $ng.mL^{-1}$ pour les autres molécules L'étude de la précision avec 3 contrôles de qualité (150/300 ; 800 ; 2200 $ng.mL^{-1}$) montrent des CV inférieurs à 10 %. L'exactitude de la méthode est satisfaisante avec des valeurs de biais toutes inférieures à 5 %, sans interférence avec la quinine et d'autres antipaludéens de synthèse La conservation du CG est bonne à toutes les températures même à long terme, tandis qu'une conservation prolongée et à haute température décroît significativement les concentrations en CQ et PG. L'application de notre méthode chez 10 patients sous Savarine®, montre que celle-ci est suffisamment sensible pour le suivi d'une prophylaxie.

Conclusion : Nous rapportons une technique fiable et sensible pour le suivi prophylactique de la Savarine® à partir de 80 µL de sang total recueilli sur spot de papier buvard. Le recueil sur DBS paraît adapté aux études pharmacologiques et/ou épidémiologiques réalisées sur le terrain dans des conditions difficiles

Mots clés : spots de sang séché, antipaludéens, CLHP

4. Détection quantitative d'éthylglucuronide dans la sueur

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Introduction : Les prélèvements de sueur destinés à des analyses toxicologiques sont généralement réalisés à l'aide de patchs semi-perméables permettant l'évaporation de l'eau mais retenant les substances qui y sont contenues et qui s'accumulent durant la période d'application du dispositif (de quelques heures à plusieurs jours). De nombreuses substances médicamenteuses et drogues illicites ont ainsi pu être analysées dans cette matrice au cours des précédentes années. Cependant, la quantité de sueur correspondant aux substances extraites du patch étant inconnue, les informations fournies demeurent semi-quantitative, ce qui représente la principale limitation au développement de l'utilisation de cette matrice.

Les travaux présentés ici visent à évaluer l'utilisation des cations (sodium et potassium) naturellement présents dans la sueur, comme étalon interne permettant de déterminer la quantité de sueur accumulée sur le patch afin de déterminer la concentration dans la sueur des substances recherchées. La méthode a ensuite été appliquée à la détermination quantitative de l'éthyle glucuronide (EtG), métabolite de l'éthanol, qui n'avait jusqu'ici jamais encore été analysé dans la sueur.

Méthodes : Des prélèvements de sueur ont été réalisés auprès de 25 volontaires à l'aide de patchs PharmCheck™ ainsi qu'avec un dispositif « home-made » permettant la collecte de sueur non évaporée. Le dosage des cations présents dans la sueur et dans les extraits de patchs a ensuite été réalisé par électrophorèse capillaire pour comparaison.

Dans un second temps, des patchs ont été appliqués sur 11 volontaires pendant une période au cours de laquelle ces derniers ont consommé une quantité variable d'alcool. Après extraction des patchs à l'eau, les cations sodium ont été dosés et l'EtG accumulé a été analysé par chromatographie gazeuse couplée à une détection par spectrométrie de masse.

Résultats : Le sodium s'est révélé être un étalon interne satisfaisant en raison de sa faible variabilité inter-individu (1039 ± 89 mg/L chez les femmes et 711 ± 45 mg/L chez les hommes). Les bons rendements d'extraction (cations et EtG) observés ont montré l'intérêt des patchs pour l'analyse quantitative de l'EtG dans la sueur. De l'EtG a été détecté dans la sueur chez tous les volontaires ayant consommé de l'alcool. La quantité d'EtG par patch, rapportée à la quantité de sodium correspondante a permis de calculer des concentration d'EtG dans la sueur s'étalant de 4,7 à 39,2 µg/L pour des quantités d'alcool consommé allant de 38 à 102 g d'équivalent éthanol pur, ces deux paramètres étant statistiquement corrélés ($r_p = 0,6903$; $p = 0,0396$).

Conclusion : Le dosage des cations sodium extraits des patchs semble être un étalon interne satisfaisant pour évaluer la quantité de sueur recueillie. D'autre part, et bien que d'autres travaux soient nécessaires avant de pouvoir évaluer précisément la quantité d'alcool consommée grâce à une analyse de sueur, cette étude démontre qu'à la suite d'une consommation d'alcool, de l'EtG peut être détecté dans la sueur collectée à l'aide d'un patch. Ceci peut représenter une alternative intéressante aux analyses d'urine et de sérum pour le contrôle de l'abstinence dans le cadre de programmes de désintoxication ou dans un cadre professionnel de médecine du travail.

Mots clés : éthylglucuronide, sueur, patch

5. Apport de la toxicologie analytique au diagnostic de l'origine « factice » d'un trouble métabolique : applications aux cas des agents hypoglycémiants et diurétiques

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Introduction : Les maladies factices, dont les symptômes sont simulés ou créés en toute connaissance de cause, peuvent revêtir des formes extrêmement variées. Parmi celles-ci, il est parfois observé des troubles métaboliques à type d'hypoglycémie ou d'hypokaliémie à répétition, dont l'origine est souvent difficile à cerner du fait de leur caractère « factice » ; ces troubles étant liés le plus souvent à la prise clandestine de composés hypoglycémiants ou hypokaliémiants. Dans ce contexte, la recherche des agents responsables dans les matrices biologiques présente un intérêt tout particulier pour le diagnostic différentiel. Nous présentons le bilan du laboratoire sur deux années (2006 - 2007) concernant les recherches de ces substances dans le sang ou les urines de patients pour lesquels une consommation clandestine et volontaire de ces médicaments était suspectée.

Méthodes : Un recueil systématique de l'ensemble des demandes de recherches de composés hypoglycémiants (sulfamides et glinides) ou diurétiques (diurétiques de l'anse principalement) adressés au laboratoire était effectué de janvier 2006 à décembre 2007. La recherche et le dosage de six sulfamides hypoglycémiants (glibenclamide, glibornuride, glicazide, glimepiride, glipizide, carbutamide) et du répaglinide étaient effectués dans le plasma après extraction liquide-liquide par l'éther en milieu acide, et analyse par LC-MS/MS à trappe d'ions en mode « full scan ». La recherche qualitative de diurétiques (notamment les diurétiques de l'anse : furosémide, bumétanide) dans les urines était réalisée sur le même système analytique après une double extraction liquide-liquide en milieu acide puis en milieu basique. Pour ces deux méthodes, la séparation chromatographique était réalisée au moyen d'une colonne Hypurity® C18 (150 x 2,1 mm ; 5 µm), et d'une phase mobile, constituée du mélange acétonitrile/acide formique 0,1 % (50/50, v/v) en mode isocratique.

Résultats : Sur la période étudiée, 150 recherches pour hypoglycémies inexpliquées et 15 pour hypokaliémies, ont été pratiquées au laboratoire à la demande de 42 services hospitaliers français, principalement des services d'endocrinologie. Dans 15 cas sur 150, des sulfamides hypoglycémiants étaient retrouvés (glibenclamide : 6, gliclazide : 4, glimépiride : 4, glipizide : 1). Les concentrations plasmatiques des composés identifiées étaient comprises dans la zone des concentrations thérapeutiques, excepté pour 2 cas où le gliclazide était retrouvé à des concentrations environ 3 et

6 fois supérieures à cette zone. Les glycémies mesurées sur ces échantillons étaient toutes inférieures à 2,2 mmol (0,4 g/L) et sans relation avec les concentrations plasmatiques des composés identifiés. Sur les 15 demandes concernant les agents diurétiques, 2 cas « positifs » au furosémide dans les urines étaient retrouvés chez des patients qui présentaient une hypokaliémie profonde (< 2 mmol) et chronique non expliquée.

Conclusion : Ce bilan souligne à nouveau tout l'intérêt de disposer de techniques analytiques permettant de confirmer ou d'infirmer le diagnostic de la consommation occulte d'agents hypoglycémiants ou hypokaliémiants dans un contexte de pathologie factice. À l'avenir, l'analyse des cheveux, plus difficile à mettre en oeuvre en raison des faibles concentrations attendues (de l'ordre du pg/mg), devrait permettre une recherche plus fine de ces troubles métaboliques inexpliqués, notamment en vue de déterminer l'origine d'épisodes anciens et répétés d'hypoglycémie ou d'hypokaliémie pour lesquels aucune explication n'a pu être donnée à l'époque.

Mots clés : hypoglycémiant, hypokaliémiant, LC-MS/MS

6. Intoxication mortelle avec une infusion de feuilles de chêne et des médicaments

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Introduction : Un jeune homme âgé de 21 ans a été admis inconscient aux urgences d'un hôpital périphérique pour une suspicion de tentative d'autolyse. Les paramètres biologiques du bilan d'entrée étaient très perturbés et ont montré une aggravation au cours de l'hospitalisation. L'anamnèse et une recherche au domicile du patient ont suggéré une prise possible de médicaments accompagnée d'une infusion de feuilles de chêne ; un bilan toxicologique nous a alors été demandé. L'état du patient s'est par la suite très rapidement dégradé et 24 h après son admission, il est décédé d'une défaillance multiviscérale.

Méthodes : Une recherche urinaire des stupéfiants (amphétamines, cannabis, cocaïne, opiacés) a été réalisée par des méthodes immunologiques (Microgenics®, Roche Modular®). Une recherche non spécifique de 1ère intention a été effectué sur les urines et le plasma en CLHP/BD (Waters®) après extraction liquide/liquide basique. Dans un 2e temps, l'acide tannique (composé des tanins du chêne) et ses métabolites (acide gallique et pyrogallol) ont été recherchés de manière spécifique sur les liquides biologiques extraits par cartouche SPE (Bond Elute Plexa®) en milieu acide. Nous avons comparé ces échantillons à des blancs surchargés par analyse qualitative type « screening » en CPG-SM (Agilent®/HP®) avec et sans dérivation, et en CL-SM (QuattroMicro avec Chromalynx®, Waters®). Compte tenu des résultats, une analyse quantitative sur Acquity/TQD (Waters®) en ionisation négative (ESI-) et mode MRM (aux transitions 124,85 > 97,1 et 79 pour le pyrogallol et 168,8 > 126,9 et 79 pour l'acide gallique) a été entreprise.

Résultats : À son arrivée, le patient présentait une acidose (pH = 6,82), une hémolyse avec existence d'une macrocytose et surtout une élévation de la créatininémie (382 µmol/L), de l'urémie (13,1 mmol/L) et des enzymes hépatiques (ASAT, ALAT et CK respectivement à 7953, 4948 et 55402 UI/L). L'analyse toxicologique réalisée en urgence a montré l'absence de toute substance stupéfiante, mais a révélé la présence à concentration thérapeutique de cyamémazine et d'oxazépam dans le plasma (148 et 728 ng/mL respectivement) et les urines. Cependant, au cours de ces premières analyses, les substances supposées provenir de l'infusion de feuilles de chêne n'ont pas été retrouvées étant probablement peu extraites en milieu basique, et absentes des bibliothèques de recherche. L'analyse qualitative par comparaison des spectres et des temps de rétention des composés a montré la présence d'un pic important de pyrogallol et d'acide gallique dans les urines et le plasma, mais pas d'acide tannique.

Conclusion : L'intoxication par ingestion de feuilles de chêne ou de glands généralement rapportée chez les bovins, ovins, chevaux ou pigeons est due à la présence d'acide tannique métabolisé en acide gallique puis en pyrogallol. À faibles doses, ces composés contenus dans les tanins ont des propriétés pharmacologiques et même antidotiques. À fortes doses, le pyrogallol (que l'on retrouve également dans des produits de développement photographique) et l'acide gallique sont responsables de la toxicité générale du chêne (et de nombreux autres végétaux !!!) qui se traduit chez les animaux par des coliques, une anorexie, un ictère ou une défaillance rénale pouvant aller jusqu'au décès. L'observation rapportée ici constitue le premier cas documenté chez l'homme d'un sujet décédé ayant potentiellement ingéré des parties végétales de chêne ; grâce à la diversité des techniques analytiques utilisées, il a été montré dans les liquides biologiques la présence de ces composés toxiques, compatible cliniquement avec l'hypothèse de l'absorption d'une infusion de feuilles de chêne.

Mots clés : pyrogallol, intoxication mortelle, spectrométrie de masse

7. Place des laboratoires de toxicologie hospitalière dans le système français de toxicovigilance

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Introduction : La toxicovigilance a été organisée pour la première fois en France par le décret 99.841 du 28 septembre 1999. Selon ce décret, la toxicovigilance a pour objet : « la surveillance des effets toxiques pour l'homme d'un produit, d'une substance, ou d'une pollution aux fins de mener des actions d'alerte, de prévention, de formation et d'information ». Cette vigilance, non statutaire à ce jour est peu connue des toxicologues analystes qui ne sont pas ou peu associés à l'organisation actuelle. Notre objectif est de présenter les propositions du rapport d'évaluation du dispositif de toxicovigilance de l'inspection générale des affaires sociales (IGAS) rendu public le 17 décembre 2007 et de décrire quelle peut être la place des laboratoires au sein d'un tel dispositif.

Présentation du rapport et discussion : Sous la direction de l'Institut de Veille Sanitaire (InVS) depuis la mi-2005, cette vigilance est exercée par dix centres antipoison et de toxicovigilance et trois centres de toxicovigilance répartis sur le territoire métropolitain (il n'existe aucun centre dans les départements et territoires d'Outre Mer). L'activité de ces centres repose essentiellement sur les alertes émanant de l'analyse des réponses téléphoniques urgentes, elle est donc surtout adaptée à la collecte d'informations concernant l'exposition aiguë. Dans leur rapport, les membres de l'IGAS proposent une orientation du dispositif de toxico vigilance vers la surveillance et l'alerte tant dans le domaine des intoxications aiguës que des intoxications chroniques et une réorganisation de ce système, sous la forme de pôles de référence interrégionaux de toxicologie au sein desquels apparaissent les laboratoires. De plus, selon les auteurs du rapport, une réflexion sur les sources de données autres que la réponse téléphonique à l'urgence, apparaît comme un préalable à l'existence d'une réelle activité de toxicovigilance. Quelle peut donc être la place des laboratoires au sein d'un tel dispositif ? Celle-ci pourrait être de trois ordres :

1- Nouvelle source de données : la création d'une banque de donnée des résultats d'analyse toxicologique hospitalière et médicolégale pourrait être proposée dans le cadre des commissions de la SFTA afin de conforter l'activité de vigilance de celles ci en liaison avec le futur système de toxico vigilance.

2- Réponse analytique aux interrogations posées par les expositions répétées aux toxiques environnementaux. Cette problématique fait l'objet d'études épidémiologiques auxquelles les laboratoires doivent être en mesure d'apporter une réponse analytique.

3- Développement de la recherche clinique et fondamentale. Les projets de recherche clinique PHRC sont en effet très peu nombreux dans le domaine de la toxicologie, l'une des raisons est sans doute la multiplicité des disciplines impliquées et la méconnaissance des « réseaux » de compétences.

Conclusion : La place des laboratoires dans le dispositif français actuel de toxico vigilance apparaît comme marginale. L'évolution nécessaire de cette place passera essentiellement par la collecte exhaustive des données analytiques hospitalières et médico-légales et par la mise en place d'un système centralisé d'informations scientifiques. Les Départements d'Outre Mer particulièrement touchés par l'exposition aux produits phytosanitaires devraient pouvoir bénéficier de ces nouvelles perspectives dans le domaine de la toxicologie.
Mots clefs : toxicovigilance.

8. Étude d'un kit de dépistage immunoenzym[illegible] des antidépresseurs tricycliques : analyse statisti[illegible]e la structure des molécules et affinité pour l'anticor[illegible]

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Introduction : Les kits immunochimiques présent[illegible]rand nombre d'interférences peu connues et généralement no[illegible]iées. Afin de déterminer quelles molécules présentaient un risqu[illegible]tion faussement positive et de quantifier cette réaction, nous av[illegible]ché à établir des proximités structurelles à l'aide d'une anal[illegible]mensionnelle des molécules réagissant avec un kit de recherche de[illegible]esseurs tricycliques (ADT).

Méthodes : Nous avons étudié le kit de d[illegible]Emit® Tox™ Serum Tricyclic Antidepressants Assay (Dade-Beh[illegible]is La Défense) utilisé sur un automate Dimension® Xpand®. U[illegible] rétrospective sur 1 an concernant 1657 tests a conduit à 426 é[illegible]ns supérieurs au zéro de l'étalonnage et explorés par d'autres [illegible] analytiques (CPG-NPD, Biorad® Remedi®, CPG-SM). Après dé[illegible]on des molécules d'intérêt, des plasmas ont été chargés puis ana[illegible] l'automate. Après calcul du pourcentage de réactivité vis-à-vis [illegible]orps, la géométrie de chaque molécule a été étudiée à l'aide [illegible]ule 3 dimensions du logiciel ChemSketch® 10.0 (ACD Labs). [illegible]res ont porté sur 6 distances et 5 angles inter-atomiques. Une an[illegible] composantes principales (ACP) a été réalisée sur n = 42 molécule[illegible] caractères.

Résultats : L'ACP a permis d[illegible]nter les molécules sur des plans de projection conservant en mo[illegible] mieux les distances entre celles-ci, en introduisant de nouveaux[illegible]es synthétiques. Différentes variables présentent une forte corr[illegible]ositive entre elles. La projection des molécules selon les 3 pla[illegible]paux (plans 1-2, 1-3 et 2-3) permet de confronter les résultats [illegible]és obtenus avec la structure moléculaire. Les ADT vrais sont rép[illegible] groupés sur les plans principaux 1-2 et 1-3, correspondant respecti[illegible]ux dibenzazépines, dibenzocycloheptadiènes et dibenzoxépines a[illegible]ux dibenzothiépines ; en revanche, ils sont regroupés sur le plan[illegible]1 2-3. Les phénothiazines sont toutes regroupées quelque soit le plan [illegible]ction (plans 1-2, 1-3 et 2-3), montrant une grande homogénéité stru[illegible] sein de la famille. Sur le plan 1-3, la maprotiline est proche des di[illegible]ines et dibenzothiépines, ce qui peut éventuellement expliquer une d[illegible]du même ordre. Sur le plan 2-3, le zuclopenthixol se retrouve proch[illegible]énothiazines et la cyproheptadine proche des ADT, ce qui peut e[illegible] son interférence au test. Pour d'autres molécules, en particulier [illegible]istaminiques et les benzodiazépines structuralement hétérogène[illegible]est pas possible de retrouver des structures communes.

Conclusi[illegible]nalyse par composantes principales a permis d'obtenir des simi[illegible]tructurelles de certaines molécules et ainsi, d'appréhender les rai[illegible]ntuelles des interférences obtenues, ainsi qu'un début de quanti[illegible]de ces interférences. Le but à terme est d'obtenir un modèle préd[illegible]ettant de passer au crible de nombreuses molécules et de dét[illegible]esquelles auront un risque d'interférence. Après validation d'un tel modèle, il serait aisé de le transposer à d'autres kits immunochimiques permettant de faire une étude fine de ces kits [illegible]s à un nombre limité de test[illegible]rme de temps et de réactifs.

Mots-clés : a[illegible]s tricycliques, immunochimie, corrélation

9. Dosage rapide et simultané de 14 neuroleptiques et autres phénothiazines dans le sang par CL-SM-SM

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Introduction : Les neuroleptiques et notamment les phénothiazines sont des molécules psychotropes pouvant être rencontrées dans les cas de toxicologie clinique et médicolégale et dont la recherche doit être entreprise lors d'une suspicion de soumission chimique. Une méthode simple et rapide a été développée pour quantifier simultanément 14 composés : niaprazine, acéprométazine, propéricyazine, prométhazine, pipotiazine, cyamémazine, alimémazine, lévopromazine, chlorpromazine, fluphénazine, olanzapine, doxylamine, rispéridone et halopéridol dans le sang. Cette méthode est basée sur une analyse par chromatographie liquide couplée à une détection par spectrométrie de masse en tandem (CL-SM-SM).

Méthode : L'extraction de l'échantillon (500 µL) est réalisée en phase liquide par un mélange acétate d'éthyle/butanol (80/20, v/v) en milieu alcalin, en utilisant la chlorpromazine deutérée (D3) comme étalon interne. Après dilution de l'extrait, la séparation des analytes est réalisée sur une colonne ThermoHypersilGold (C18, 3 µm, 10 x 2.1 cm) à l'aide du mélange acétonitrile/acide formique 0,1 % en gradient. La détection est assurée par un détecteur SM/SM (Quantum Discovery Plus, Thermofisher) en mode d'ionisation positive par électrospray. La quantification, basée sur un mode MRM, est réalisée au moyen de gammes étalons préparées dans les mêmes conditions.

Résultats : La méthode a été validée pour la matrice sanguine et les rendements d'extractions sont variables selon la molécule. La durée totale de l'analyse est de 14 minutes. L'effet de suppression d'ion n'intervient pas aux temps de rétention des analytes concernés.

Molécule	Concentrations Thérapeutiques (mg/L)	LOQ (mg/L)	RT (min)	Ion Parent	Ion Fils
Olanzapine	0,01 - 0,05	0,005	1,70	313	256
Doxylamine	0,05 - 0,2	0,001	5,00	271	167
Niaprazine		0,020	8,20	357	177
Rispéridone	0,01 - 0,03	0,001	9,40	411	191
Acéprométazine	0,1 - 0,4	0,005	11,00	327	240
Halopéridol	0,005 - 0,015	0,001	11,10	376	123
Propéricyazine	0,005 - 0,05	0,001	11,15	366	142
Prométhazine	0,1 - 0,4	0,005	11,25	285	198
Pipotiazine	0,001 - 0,06	0,005	11,50	476	170
Cyamémazine	0,05 - 0,4	0,005	11,70	324	100
Alimémazine	0,05 - 0,4	0,020	11,80	299	100
Lévopromazine	0,03 - 0,15	0,015	12,00	329	100
Chlorpromazine	0,03 - 0,5	0,005	12,45	319	86
Fluphénazine	0,001 - 0,017	0,005	12,50	438	171

Les limites de quantification sont de l'ordre de 0,001 à 0,02 mg/L selon la molécule analysée ce qui représente une sensibilité de l'ordre de 1 à 20 fois inférieure à la concentration thérapeutique basse de la molécule concernée.

Conclusion : La rapidité et la sensibilité de cette méthode permettent d'envisager son application non seulement dans le domaine de la toxicologie clinique mais aussi dans le cadre de la soumission chimique.

Mots clés : neuroleptiques, phénothiazines, LC-MS/MS

10. À propos d'un cas d'intoxication massive au topiramate

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Introduction : Nous rapportons un cas d'intoxication massive au topiramate, médicament anti-épileptique indiqué dans les épilepsies partielles et les crises généralisées tonico-cloniques. Un homme de 57 ans, ayant des antécédents dépressifs, est retrouvé inconscient par sa famille. L'état comateux est profond, avec d'emblée un glasgow à 3, nécessitant une ventilation mécanique assistée. Le diagnostic évoqué lors de la prise en charge du patient, est une crise de grand mal épileptique, compliquée d'une embolie pulmonaire au vu des images tomodensitométriques. Un électroencéphalogramme effectué 48 heures après son admission, montrait un tracé surchargé d'artefacts avec signes de souffrance cérébrale diffuse. Ce patient épileptique est traité par du topiramate (Epitomax®), et de l'oxcarbazépine (Trileptal®). Des prélèvements biologiques ont été réalisés pour effectuer des analyses toxicologiques chez ce patient.

Méthodes : Les dosages plasmatiques du topiramate et de l'oxcarbazépine ont été réalisés par une technique de LC-MS/MS avec une préparation en ligne des échantillons. Après ajout de l'étalon interne (zolpidem deutéré), l'échantillon est dilué au 1/100ème dans l'eau. 50 µL sont injectés dans système HPLC Alliance 2796 (Waters®) couplé à un spectromètre de masse Quattro Micro (Waters®). La séparation chromatographique est réalisée sur une colonne Phenomenex Luna 5 µ phenyl-hexyl (50 x 2,0 mm ; 5 µm), avec un gradient acétonitrile-formate d'ammonium 5 mM (pH = 3,5). L'acquisition s'effectue en mode MRM avec les transitions suivantes : 357,1 > 254,0 (topiramate), 253,1 > 180,2 (oxcarbazépine), 255,2 > 194,1 (10-OH-carbazépine), 314,3 > 263,5 (zolpidem deutéré).

Résultats : La concentration plasmatique du topiramate est de 77 µg/mL dans le prélèvement effectué lors de la prise en charge du patient aux urgences, environ 14 heures après l'ingestion du médicament. Les concentrations thérapeutiques se situeraient entre 3,4 et 5,2 µg/mL. Un second prélèvement a été effectué 72 heures après le premier dosage, la concentration plasmatique est de 9,6 µg/mL. Les concentrations plasmatiques de l'oxcarbazépine dans ces deux échantillons sont respectivement de 3,4 et 3,0 µg/mL ; les concentrations du métabolite, 11-OH-carbazépine, sont respectivement de 76,3 et 91,3 µg/mL ; ces concentrations sont d'ordre thérapeutique.

Conclusion : Le topiramate est un anti-épileptique (monosacharide sulfamate-substitué) qui augmente l'activité du neurotransmetteur GABA. Il ne semble pas y avoir de corrélation entre les concentrations plasmatiques et l'efficacité thérapeutique. La toxicité du topiramate est essentiellement neuro-psychique (confusion, somnolence, ataxie, hallucinations…). La pharmacocinétique du topiramate est peu décrite dans le cas d'une intoxication massive. Il semble que 90 % de la dose ingérée soit éliminée après 3 à 4 demi-vies ($T_{1/2}$ = 20 à 30 h). Ce cas d'intoxication massive au topiramate, à l'origine d'un coma profond, hypotonique, a connu une évolution favorable avec une récupération progressive d'une vigilance satisfaisante. L'extubation surviendra 7 jours après l'admission en réanimation. Les cas d'intoxication massive au topiramate restent peu fréquents. La sévérité de ces intoxications est souvent modérée ; les cas mortels ou associés à des séquelles persistantes sont très rares.

Mots clés : topiramate, intoxication massive, LC-MS/MS

11. Intoxications par le lithium : intérêt du dosage du lithium plasmatique dans l'évaluation et la prise en charge clinique

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Introduction : Le lithium (Li) est utilisé dans le traitement des troubles bipolaires. Du fait d'un faible index thérapeutique, les intoxications par le Li sont souvent graves. Le dosage plasmatique est alors primordial dans l'évaluation de la gravité de l'intoxication pour permettre la mise en place rapide d'une thérapeutique épuratrice salvatrice. Nous présentons deux cas cliniques afin d'illustrer l'importance du dosage rapide du Li plasmatique.

Méthodes : La méthode utilisée pour le dosage du Li plasmatique dans le cas 1 et pour le dosage du Li érythrocytaire dans le cas 2 sont des méthodes classiques en spectrométrie d'absorption atomique sur un appareil de type Spectra 200 (Varian). Dans le cas 2, une nouvelle méthode spectrophotométrique (Infinity™, ThermoFisher Scientific) adaptée à un automate de type Hitachi 912 a été utilisée. La mesure est réalisée à 480 nm après réaction du Li avec une porphyrine en milieu alcalin. Cette méthode de dosage rapide, linéaire de 0,11 meq /L à 3 meq/L est actuellement utilisée 24h/24 au CHRU de Lille.

Résultats : Cas 1 : au décours d'une pneumopathie fébrile, un homme de 50 ans est hospitalisé pour un syndrome confusionnel compliqué d'une crise convulsive. Le dosage du Li plasmatique sur un prélèvement réalisé aux urgences permet de poser rapidement le diagnostic de surdosage accidentel au Li (Li plasmatique = 15,68 meq/L) et de placer sans délai le patient sous hémodialyse prolongée.

Cas 2 : une femme de 50 ans ingère volontairement 70 comprimés de Téralithe® LP 400 mg. Elle consulte rapidement aux urgences où est pratiqué un lavage gastrique et où on lui administre du charbon activé. Le Li plasmatique est mesuré 4 heures après l'ingestion à 2,44 meq/L avec une fonction rénale normale (créatinine sanguine = 9 mg/L). Les heures suivantes seront marquées par l'apparition de troubles digestifs, d'une dégradation de l'état neurologique nécessitant une intubation et une ventilation contrôlée et d'une insuffisance rénale aiguë (créatinine sanguine = 24 mg/L). Malgré le lavage gastrique et l'administration de charbon activé, les concentrations plasmatiques de Li vont augmenter jusqu'à 9,8 meq/L à la 12ème heure après l'ingestion. Une épuration extra-rénale est alors mise en place en urgence. La patiente va secondairement présenter une défaillance hémodynamique imposant la mise sous noradrénaline pendant 48 heures. Après 72 heures d'épuration extra-rénale continue, les concentrations en Li plasmatique se normalisent. L'évolution de ces concentrations (meq/L) est décrite dans le tableau suivant :

	j = 1 t = 4 h	j = 2 t = 12 h	j = 3 matin	j = 3 après midi	j = 4 matin	j = 4 après midi	j = 6
Li plasma	2,44	9,8	5,7	11	2,18	1,13	0,60
Li érythrocytaire				6,9			

Conclusion : Dans ces deux cas, les deux patients ont survécu à des intoxications massives par le Li avec une concentration plasmatique mesurée élevée (15,68 mEq/L) jamais décrite jusqu'à présent chez un intoxiqué survivant. Compte tenu des nombreuses circonstances à l'origine de surdosage par le Li, il est primordial que le dosage plasmatique soit fait rapidement chez tout patient traité par Téralithe® se présentant dans un service d'urgence pour une altération de l'état général, une infection fébrile, une atteinte neurologique ou une tentative de suicide.

Mots clés : lithium, intoxication aiguë, urgence

12. La carambole (*Averrhoa carambola* L.), un fruit tropical susceptible de provoquer des intoxications

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Objectif : La carambole est le fruit comestible d'une plante tropicale, le carambolier (*Averrhoa carambola* L.), de la famille des Oxalidaceae. D'origine asiatique, on peut considérer de nos jours que cette plante est devenue pantropicale. De nombreuses observations liées à la toxicité rénale ou neurologique de la carambole peuvent être retrouvées dans la littérature, toutes font état d'une toxicité aiguë chez les patients atteints d'une affection rénale sévère ou terminale. Une revue de la littérature, la présentation clinique de cas, les hypothèses de toxicité et des propositions de prise en charge thérapeutique sont présentées.

Matériel et méthodes : Etude rétrospective et présentation de cas de patients hospitalisés au CHU de Pointe à Pitre pour intoxication par la consommation de carambole.

Résultats : Depuis 1998, une trentaine de cas d'intoxication par la carambole ont été recensés au CHU de Pointe à Pitre chez des patients présentant une atteinte de la fonction rénale. Toutefois nous rapportons également plusieurs cas d'intoxication aiguë chez des patients non connus pour leur insuffisance rénale mais porteurs d'une altération glomérulaire ou tubulo-interstitielle objectivée rétrospectivement par une biopsie rénale. La sévérité de l'intoxication n'apparaît pas dépendante du degré de l'affection rénale préexistante et du nombre de fruits consommés. Une classification des cas en trois niveaux avec une conduite thérapeutique adaptée a été proposée : (i) intoxication légère : hoquet, vomissements et insomnie ; (ii) intoxication modérée : agitation psychomotrice, paresthésies, faiblesse musculaire, légère confusion mentale ; (iii) intoxication sévère : confusion mentale modérée à sévère évoluant vers un coma, convulsions, état de mal épileptique et instabilité hémodynamique pouvant générer une hypotension et un choc. Les manifestations biologiques sont évocatrices d'une insuffisance rénale aiguë, ce sont : l'acidose métabolique et l'élévation de la créatinine sanguine jusqu'à 500 à 1000 µmol/L ou une majoration de plus de 200 µmol/L chez les insuffisants rénaux connus. L'anamnèse permet le plus souvent de faire le diagnostic car les analyses toxicologiques des échantillons biologiques ne mettent en évidence qu'une augmentation de l'acide oxalique urinaire. Récemment l'existence d'une fraction neurotoxique a été mise en évidence dans le fruit de la carambole.

Conclusion : Autrefois réduite aux régions tropicales, la commercialisation de la carambole est de plus en plus répandue en Europe. La consommation de manière exagérée ou par des personnes atteintes d'affections rénales du fruit ou de jus de fruits non dilué de la carambole peut se traduire par une intoxication plus ou moins sévère. La prise en charge doit être symptomatique avec recherche d'une affection rénale et maintient en observation du patient sur plusieurs jours. Dans le cas des patients insuffisants rénaux dialysés il faudra avoir recours systématiquement à la dialyse qui devra être répétée jusqu'à disparition des symptômes. La gravité de l'intoxication doit conduire les médecins traitants et les diététiciens à interdire la consommation de ce fruit chez les insuffisants rénaux.

Mots clés : carambole, toxicité

13. Le chlordécone : un profil toxicologique particulier

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Le chlordécone est un insecticide organochloré employé aux Antilles françaises jusqu'en 1993 pour lutter contre le charançon du bananier. Ses caractéristiques physico-chimiques ainsi que son extrême résistance à la dégradation biotique et abiotique expliquent sa persistance dans les soles bananières. Cette pollution a entraîné une contamination des eaux de consommation et de certaines ressources alimentaires, notamment les légumes racines.

De nombreuses informations sont disponibles concernant la dangerosité du chlordécone. Décrites de manière détaillée dans la littérature scientifique internationale, elles proviennent en partie de l'observation des conséquences d'une exposition chez les employés d'une usine fabricant le chlordécone à Hopewell (Virginie, USA). Cette exposition, qui s'est étalée de 1966 à 1975, est à l'origine du *Kepone syndrome*. Ce syndrome se caractérisé par des atteintes neurologiques (tremblements des membres, incoordination motrice, troubles de l'humeur et de la mémoire récente) et testiculaires (modifications de certaines caractéristiques spermatiques). Le délai d'apparition des troubles cliniques ainsi que leur sévérité sont corrélés à la charge corporelle estimée par la mesure de la concentration plasmatique en chlordécone. Le seuil minimal à partir duquel les signes ou symptômes se manifestent a été estimé à ~1 mg/L. Après arrêt de l'exposition, la charge corporelle en chlordécone diminue et les manifestations cliniques s'amenuisent. Au terme d'un suivi sur une dizaine d'années, les manifestations cliniques avaient complètement disparues dans la plupart des cas tout comme le chlordécone devenu indétectable dans le sang. Aucun autre trouble majeur, tumoral ou non tumoral, ne fut signalé au cours de ce suivi.

Le chlordécone est facilement absorbée par voie orale. Transporté par l'albumine et les lipoprotéines HDL, il s'accumule préférentiellement dans le foie et dans une moindre mesure dans les graisses périphériques. Après réduction par la chlordécone réductase (AKR1C4) en chlordécone alcool et glucuroconjugaison partielle, il est excrété dans la bile. Néanmoins, et avant son élimination par les selles, une fraction non négligeable subit une recirculation entéro-hépatique. La demi-vie du chlordécone plasmatique a été estimée à 165 jours.

Chez l'animal, l'exposition par voie orale au chlordécone entraîne des effets neurologiques et testiculaires similaires à ceux observés chez l'homme. L'exposition des femelles gestantes entraîne des atteintes du développement intra-utérin et du développement neurocomportemental chez la portée. En dépit d'une absence de génotoxicité, le chlordécone induit la survenue d'adénocarcinomes hépatiques chez le rat et la souris, justifiant ainsi son classement par l'IARC – OMS comme cancérogène possible pour l'homme. Des études *in vitro* et *in vivo* ont montré que le chlordécone présente une activité hormonale se traduisant par des effets génomiques et non-génomiques de type œstrogénique. Cette propriété pourrait expliquer en partie ses effets reprotoxiques. Les effets neurologiques du chlordécone pourraient être expliqués par sa capacité à inhiber diverses ATPases membranaires et à interférer avec la sécrétion de divers neurotransmetteurs.

Mots clés : chlordécone, toxicologie, perturbateur endocrinien

14. Évaluations des risques liés à l'exposition chronique au chlordécone

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Introduction : Aux Antilles, l'utilisation du chlordécone par les ouvriers agricoles de la banane (jusqu'en 1993) ainsi que la contamination des eaux destinées à la consommation (jusqu'en 2000) et de certaines denrées alimentaires sont sources d'interrogations concernant leurs effets sur la santé des populations.

Méthodes : Afin d'évaluer les risques sanitaires d'une telle pollution environnementale, des mesures d'expositions ont été réalisées moyennant le dosage du chlordécone et autres polluants persistants dans diverses matrices : sang (hommes adultes comprenant des ouvriers agricoles de la banane, femmes enceintes et cordon), lait et graisse abdominale maternelles.

Résultats : Le chlordécone, détecté dans près de 90 % des prélèvements sanguins, est le polluant organochloré le plus fréquemment identifié. Les concentrations médianes s'étalent de 0,7 ng /mL (cordon) à 6,3 ng/mL (ouvriers agricoles de la banane) et se situent à des valeurs très inférieures (deux ordres de grandeur au minimum) à celles ayant entraîné des effets lors de l'exposition industrielle qui s'est produite à Hopewell en 1975. Conformément aux données de la littérature, le chlordécone a été retrouvé faiblement accumulé dans les graisses périphériques et très peu mobilisé par l'allaitement. Une étude menée sur la fertilité des ouvriers agricoles de la banane n'a pas montré de corrélations entre la charge corporelle en chlordécone, évaluée par sa concentration plasmatique, et divers paramètres spermatiques et hormonaux. Ce résultat est en accord avec les observations antérieures montrant que les anomalies spermatiques n'apparaissent qu'à partir d'une concentration plasmatique en chlordécone de l'ordre de 1000 ng/mL. Toutefois, des incertitudes demeurent quand à l'impact que les niveaux d'expositions au chlordécone constatées de nos jours pourraient avoir sur le développement intra-utérin et postnatal. La cohorte TIMOUN (1200 femmes enceintes et 200 nouveau-nés) a comme objectif d'évaluer l'impact des expositions prénatales aux polluants organochlorés, dont le chlordécone, sur le déroulement de la grossesse, le développement prénatal et le développement neurologique postnatal. Par ailleurs, tenant compte du caractère cancérogène du chlordécone (2b CIRC-OMS) et de ses propriétés œstrogéniques, l'étude KARUPROSTATE (600 cas et 600 témoins) vise à étudier le rôle des expositions au chlordécone dans la survenue du cancer de la prostate.

Conclusion : Ces études menées actuellement en Guadeloupe permettront d'apporter des éléments complémentaires au dispositif d'évaluation des risques des pesticides, en particulier le chlordécone, mise en place aux Antilles françaises par les autorités sanitaires.

Mots clés : chlordécone, évaluation du risque, cancer prostate

15. Caractérisation des groupes de population à risque d'exposition vis à vis de la chlordécone via l'alimentation (Martinique, 2006)

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Introduction : Le chlordécone est un insecticide organochloré qui a été largement utilisé aux Antilles dans les plantations de bananes pendant une vingtaine d'années. Il a été interdit en 1993 mais depuis 1999, des enquêtes ont successivement mis en évidence sa présence dans les sols et les sédiments, les produits végétaux, les viandes et poissons. S'est alors posée la question de l'exposition de la population martiniquaise à travers l'alimentation et de la caractérisation de groupes de population à risque d'exposition au chlordécone.

Matériel et méthode : Les données de consommations alimentaires de l'enquête ESCAL et les données de contamination des aliments provenant des différents plans de contrôle des services administratifs ont permis à l'AFSSA de calculer sous diverses hypothèses le niveau d'exposition de 1495 personnes inclues dans l'enquête ESCAL (Enquête en population générale par tirage au sort, après stratification sur l'appartenance à la zone contaminée ou non, de 165 îlots de l'INSEE, puis 5 foyers tirés au sort dans chaque îlot incluant tous les membres du foyer résidant depuis plus de 12 mois en Martinique et âgés d'au moins 3 ans). Une personne à risque d'exposition a été définie comme étant une personne dont l'exposition calculée dépasse la valeur toxicologique de référence (VTR) chronique de 0,5 µg/kg pc/j (microgramme par kilogramme de poids corporel par jour). Une analyse univariée a été menée pour comparer les caractéristiques entre les individus exposés et les non exposés. Une analyse multivariée a permis de quantifier les facteurs de risque d'exposition.

Résultats : Les individus pour lesquels le calcul d'exposition dépasse la VTR se situent tous dans la zone contaminée où ils représentent 21 % chez les enfants (moyenne 0,335 ± 0,047 µg/kg pc/j) et 16 % chez les adultes (moyenne 0,269 ± 0,020 µg/kg pc/j). Les individus à risque ne diffèrent pas selon le sexe et l'âge. Dans la zone contaminée, la proportion des individus à risque diffère significativement selon le niveau socio-économique ($p = 10^{-3}$). L'autoconsommation de légumes racines est sur le plan quantitatif le principal facteur prédictif du risque d'exposition.

Discussion : Les études menées en collaboration par l'AFSSA et la Cire Antilles-Guyane ont permis de caractériser la population à risque d'exposition élevée au chlordécone via l'alimentation. Cette population, compte tenu de ses modes d'approvisionnement, ne peut pas être protégée par des mesures réglementaires telles que des limites maximales. Sur la base de ces résultats, la Direction de la Santé et du Développement Social de Martinique met en place un programme de santé spécifique en direction des populations concernées. Ce programme est actuellement en phase d'expérimentation.

Mots clés : chlordécone, population à risque, alimentation

16. Exposition chronique aux organophosphorés, revue des aspects analytiques

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Objectif : Les organophosphorés (OP) sont des insecticides caractérisés par une rapide biodégradation (pesticides non persistants) qui a conduit à leur usage extensif malgré l'existence d'un risque d'intoxication aiguë. Leur omniprésence dans l'environnement se traduit par l'exposition chronique de la population générale. Depuis dix ans, le lien entre cette exposition et une éventuelle pathologie fait l'objet d'études. Devant le manque de sensibilité de la mesure de l'activité des acétylcholinesterases globulaires, biomarqueur d'effet, il est désormais nécessaire d'avoir recours au dosage des métabolites urinaires, les dialkylphosphates (DAP). Nous avons pour objectif de présenter les différentes difficultés pré-analytiques et analytiques liées à ce dosage.

Matériel et méthode : Une revue de la littérature concernant la pertinence de la collecte des échantillons biologiques et les méthodes analytiques sera présentée.

Résultats : La mise en évidence épidémiologique d'une toxicité par exposition à des doses faibles et répétées se heurte à d'importantes difficultés méthodologiques. La question de la nature et du mode de recueil des échantillons biologiques est une de celles ci. Les OP sont caractérisés par la rapidité de leurs métabolismes [demi-vie : 0,29 h à 3,62 h] et l'apparition de métabolites urinaires communs appelés non spécifiques. Le dosage des composés natifs dans le sérum implique de faire appel à des techniques très sensibles (ng/L) et coûteuses. Aussi, il est d'usage de recourir au dosage des métabolites urinaires. La limite principale de l'analyse urinaire des DAP est la variabilité temporelle de l'exposition chronique et donc de la représentativité du recueil. La mesure de l'exposition ne peut être réalisée sur un échantillon journalier unique. Le recueil des urines de 24 h étant extrêmement contraignant, pour pallier aux variations intra et inter jour, il est conseillé de faire deux recueils par jour : matin et soir et ceci séquentiellement sur plusieurs jours par mois. L'ajustement du résultat à la valeur de la créatinine est discuté du fait de la variation avec l'âge ou l'ethnie. L'interprétation de la mesure des métabolites constitue également un problème tant qualitatif que quantitatif. En effet, la mise en évidence des DAP est significative d'une exposition de classe (OP) et leur quantification pourrait ne pas être due uniquement au métabolisme chez l'homme, mais également à l'exposition de ce dernier à des DAP déjà présents dans l'environnement. Le recueil biologique doit toujours être associé à un questionnaire relatif aux habitudes alimentaires et aux éventuelles expositions environnementales. De nombreuses méthodes de dosage sont rapportées dans la littérature. Les DAP étant des composés très polaires, plusieurs méthodes d'extraction

ont été étudiées : liquide/ liquide avec un solvant polaire, cartouches SPE, distillation azéotropique, lyophilisation. La détection et la quantification des DAP est généralement réalisée par chromatographie en phase gazeuse couplée à différents détecteurs sélectifs (FID, NPD, MS, MS/MS). La limite de détection est de l'ordre du µg/L. L'analyse par chromatographie en phase liquide couplée à la spectrométrie de masse tandem est décrite avec une limite de quantification équivalente.

Conclusion : Le dosage des métabolites urinaires des OP constitue selon toute vraisemblance, un biomarqueur d'exposition accessible aux techniques courantes, mais ne fait pas encore l'objet d'un véritable consensus.

Mots clefs : organo-phosphorés, exposition chronique, dosage.

17. La réversion des effets respiratoires du paraoxon : origine centrale ou périphérique ?

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Introduction : Les organophosphorés sont responsables chaque année de millions de cas d'intoxications à travers le monde avec une mortalité voisine de 10 pour cent. S'il est parfaitement établi que la mortalité est liée à la toxicité respiratoire de ces composés, le mécanisme, central ou périphérique, des effets respiratoires reste encore à préciser. Nous avons préalablement montré, que le diéthylparaoxon (PO) induit des troubles respiratoires qui sont totalement corrigés par une seule injection d'atropine. Le but de ce travail est de préciser l'origine centrale ou périphérique, en comparant l'action de l'atropine à celle de la méthylatropine, composé incapable de franchir la barrière hémato-encéphalique.

Méthodes : Les rats mâles Sprague-Dawley (250 - 350 g) ont été intoxiqués par injection sous cutanée de PO à la dose de 0,215 mg/kg (50 % de la DL_{50}). Trente minutes après l'injection de PO, ils ont reçus par voie sous-cutanée de l'atropine (10 mg/kg sulfate), ou de la méthylatropine à dose équimolaire (0,542 mg/kg nitrate) ou supérieures (54,2 et 542 mg/kg). La ventilation au repos a été enregistrée par pléthysmographie chez l'animal vigil. Les paramètres respiratoires ont été déterminés à l'aide du logiciel Elphy (CNRS, Gif-sur-Yvette). La température centrale des animaux a été enregistrée en continue par télémétrie infra-rouge (DSI system) Tous les résultats sont exprimés en moyenne ± SEM et ont été comparés statistiquement par un test statistique ANOVA avec $p < 0,05$ comme seuil de significativité.

Résultats : L'injection de PO entraîne une diminution significative de la température 30 minutes après l'injection, qui va persister durant les 90 minutes de l'observation. Cet effet est corrigé partiellement par l'injection d'atropine mais pas par celle de méthylatropine. Sur le plan respiratoire, le PO induit une baisse de la fréquence, un allongement du temps expiratoire et une augmentation du volume courant. En comparant les doses équimolaires, l'atropine reverse complètement les effets respiratoires du PO mais pas la méthylatropine. Des doses de méthylatropine égales à 10 (54,2 mg/kg) et 100 fois (542 mg/kg) la dose équimolaire sont également sans effet sur la toxicité respiratoire du PO.

Conclusion : En traversant la barrière hémato-encéphalique l'atropine peut induire des effets muscariniques centraux et périphériques, contrairement à la méthylatropine qui ne possède qu'une action périphérique, comme le prouve l'évolution sous atropine de la température centrale des animaux intoxiqués. Sur le plan respiratoire, la méthylatropine n'apporte aucune amélioration contrairement à l'atropine. Ces résultats nous permettent de conclure que la toxicité respiratoire du diéthylparaoxon est d'origine centrale médiée par les récepteurs muscariniques.

Mots clés : paraoxon, atropine, méthylatropine

18. Exposition au méthylmercure des populations de la Guyane française

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Introduction : Depuis de longue date, il existe en Guyane une contamination environnementale par le mercure qui est directement ou indirectement liée aux activités d'orpaillage. Dans des milieux aquatiques peu oxygénés et riches en matière organique, le mercure est transformé par les bactéries en méthylmercure et il entre dans la chaîne alimentaire. La population consommant des poissons est ainsi exposée au méthylmercure. Sous cette forme, et pour ce type d'exposition chronique, le mercure est neurotoxique. Il est aussi fœtotoxique et tératogène pour de fortes doses (effets cliniques observés à Minamata pour des concentrations > 50 - 100 µg/g de cheveux). Il était donc important d'estimer et de caractériser les niveaux d'exposition des populations de Guyane, et d'identifier les déterminants de cette exposition.

Méthode : Six enquêtes ont été menées entre 1994 et 2004. Il s'agit d'enquêtes de prévalence réalisées selon un sondage de type aréolaire. Un questionnaire a été administré en face à face pour les adultes et aux parents pour les enfants de moins de 16 ans, et une mèche de cheveux a été prélevée afin de doser le mercure total. Une spéciation du mercure était généralement réalisée sur un sous échantillon de mèches de cheveux. Les analyses ont été effectuées en absorption atomique à vapeur froide après digestion des échantillons soit dans l'acide nitrique concentré (Hg total) soit dans la soude concentrée (Hg inorganique). Les données ont été analysées sous Stata, SAS et Splus.

Résultats : En prenant comme valeur de référence la concentration de mercure de 10 µg/g de cheveux (recommandée par l'OMS) le problème sanitaire est, sur le plan géographique, principalement circonscrit au Haut Maroni (65 % des adultes et 47 % des enfants ont des valeurs > 10 µg/g) et au Haut Oyapock (17 % des adultes et 13 % des enfants ont des valeurs > 10 µg/g). En terme de populations, ce sont avant tout les Amérindiens qui sont concernés, mais aussi les populations isolées des « écarts » et les populations socio-économiquement défavorisées vivant dans les bourgs. Dans ces populations, le 95ème percentile de la distribution est inférieur à 20 µg/g de cheveux (la valeur maximale observée est de 31 µg/g de cheveux). La consommation de poisson de fleuve, en particulier de carnivores prédateurs, et la faible diversité alimentaire sont les principaux facteurs contributifs de l'imprégnation mercurielle. En dehors de ces populations, les niveaux d'imprégnation de la population varient en moyenne entre 1,5 et 3 µg/g de cheveux.

Conclusion : Alors que les craintes sociales vis-à-vis du risque de malformations liées au méthylmercure sont très importantes et régulièrement alimentées par les media, la situation rencontrée en Guyane est très éloignée, du point de vue de l'exposition, de celle rencontrée à Minamata. Pour autant, il n'en reste pas moins vrai qu'une prévention ciblée sur les femmes en âge de procréer et les femmes enceintes doit être mise en place afin de prévenir les risques concernant le développement psychomoteur des enfants.

Mots clés : méthylmercure, population amérindienne, prévention

19. Validation du dosage du mercure plasmatique par ICP-MS et concentrations usuelles

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Introduction : Le dosage du mercure plasmatique par couplage inductif relié à un détecteur de masse (ICP-MS) est délicat en raison de la faible ionisation de l'élément et d'un « effet mémoire » prononcé. Nous présentons une technique de validation par ICP-MS. Les concentrations usuelles de mercure, mais également de 32 éléments dans le plasma et le sang total, sont établies à partir de prélèvements réalisés dans les nouveaux tubes pour éléments traces, chez 53 sujets volontaires.

Méthodes : L'ICP-MS est un spectromètre X7CCT Thermo Elemental (Thermo Optek, Courtaboeuf, France), sans cellule dynamique de réaction, équipé d'un passeur d'échantillon et du logiciel PlasmaLab. L'eau utilisée est purifiée extemporanément sur Milli-QPLUS 185 (Millipore, St Quentin en Yvelines, France). Les réactifs, de qualité suprapur pour analyses de traces et les solutions étalons de métaux proviennent de chez Merck (Darmstadt, Allemagne), Prolabo WWR (Fontenay/bois, France) et CPI (Amsterdam, Hollande). Des prélèvements sanguins sont effectués, chez 53 volontaires des deux sexes, indemnes de toute affection, dans des tubes en polyéthylène téréphtalate (PET) pour éléments traces de 4 mL (BD diagnostics, Le Pont de Claix, France). Les dosages de mercure dans le plasma sont réalisés à l'aide d'un étalonnage aqueux et de conditions analytiques spécifiques. Pour les 32 autres éléments dans le plasma et les 33 éléments dans le sang total, une procédure déjà décrite est utilisée. La qualité des résultats obtenus est régulièrement vérifiée par la participation au programme de comparaisons interlaboratoires par ICP-MS (Sainte Foy, Canada) et pour le mercure par l'emploi de plasma de contrôle (Seronorm, Sero, Billingstad, Norvège).

Résultats : Le coefficient de régression obtenu lors de la validation du 202Hg plasmatique est supérieur à 0,999. Les limites de détection et de quantification respectives sont de 0,02 et 0,08 µg/L. Pour cet élément, la répétabilité et la reproductibilité s'établissent à 4,4 et 7,7 %. La précision analytique est de 107,5 %. L'intervalle de référence du 202Hg est de 0,19 à 1,85 µg/L, du 5ème au 95ème percentile, dans le plasma des 53 volontaires. L'intérêt du dosage du mercure plasmatique comme marqueur biologique du mercure minéral issu des amalgames dentaires alors que le mercure sanguin total reflète essentiellement l'apport alimentaire de mercure organique sous forme de méthylmercure est précisé. Les concentrations usuelles obtenues pour les 33 éléments dans le plasma et le sang total prélevé dans les nouveaux tubes pour éléments traces chez les 53 sujets volontaires sont rapportées.

Conclusion : Cette nouvelle application confirme le potentiel et la flexibilité exceptionnels de l'ICP-MS.

Mots clés : ICP-MS, mercure, plasma

20. Dosage du cadmium urinaire : premiers résultats d'une étude multicentrique

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Introduction : Le cadmium est un toxique très cumulatif dont la demi-vie biologique varie entre 10 et 30 ans. Le dosage du cadmium dans les urines est un bon reflet de l'exposition chronique et de la charge corporelle. Il semble que pour des suivis épidémiologiques de l'imprégnation au cadmium en santé environnementale, ce dosage pose certains problèmes analytiques notamment en terme de sensibilité. En collaboration avec l'Institut de Veille Sanitaire (InVS), une étude multicentrique est réalisée par le groupe de travail « Toxiques Industriels » de la SFTA afin de faire l'état de la situation des méthodes de dosage du cadmium urinaire dans nos laboratoires et si besoin de les améliorer pour pouvoir répondre aux mesures des concentrations les plus faibles.

Méthodes : Cette étude a été réalisée dans 13 laboratoires. Trois pool d'urines (Cd_{Low}, Cd_{Medium}, Cd_{High}) ont été constitués à différentes concentrations inférieures à 0,5 µg/L (prélèvements et indication des concentrations fournis par l'InVS). A partit du pool Cd_{Low}, deux dilutions ont été réalisées (au 1/2 et au 1/4) permettant d'obtenir deux pools de très faibles concentrations. Enfin, un dernier pool (Cd_W) de concentration plus élevée a été constitué à partir d'échantillons d'urines obtenus dans le cadre de surveillances professionnelles de salariés exposés au cadmium. Les quatre échantillons et les deux dilutions ont été dosés 10 fois dans une même série par chaque participant, pour mesurer la limite de quantification (Ldq) à l'aide des CV (exprimés en %) calculés pour chaque niveau de concentration. Parallèlement, les laboratoires ont analysé 20 fois le blanc de leur méthode pour le calcul de la limite de détection (Ldd, 3 ET_{blanc}) et de la Ldq (10 ET_{blanc}). Les méthodes utilisées sont la spectrométrie d'absorption atomique électrothermique (SAAE, n = 5) et la spectrométrie de masse couplée à un plasma induit par haute fréquence (ICP-MS, n = 9).

Résultats : Les moyennes des CV intra-laboratoires sont pour les échantillons $Cd_{dil1/4}$, $Cd_{dil1/2}$, Cd_{Low}, Cd_{Medium}, Cd_{High}, et Cd_W respectivement de 18,2 %, 23,3 %, 8,6 %, 7,1 %, 6,2 % et 2,2 %. Les CV inter-laboratoires toutes méthodes confondues sont supérieurs à 20 %. Les Ldq des blancs réactifs sont mesurées entre 0,002 et 0,154 µg/L.

Conclusion : A l'exception des deux dilutions, les moyennes des CV intra-laboratoires sont inférieures à 10 %. Les moyennes des CV inter-laboratoires plus élevées montrent qu'il existe une certaine variabilité entre les laboratoires pour un même dosage alors que l'on observe des variabilités intra-laboratoires satisfaisantes pour la majorité d'entre eux. D'autre part, avec des CV de l'échantillon $Cd_{dil1/2}$ supérieurs à 20 %, on peut penser que la Ldq doit être supérieure à la valeur correspondante à la moyenne de l'ensemble des valeurs tous laboratoires réunis pour ce pool c'est-à-dire 0,077 µg/L. La mesure de la Ldq par la méthode des blancs, reste quant à elle variable entre les laboratoires avec des valeurs toujours plus élevée en SAAE. Ces premiers résultats montrent qu'un travail complémentaire en vue d'améliorer les CV inter-laboratoires est à envisager. Cette amélioration pourrait passer par l'homogénéisation des méthodes.

Mots clés : cadmium urinaire, environnement

21. Évaluation des biomarqueurs urinaires d'exposition au benzène : corrélation avec le benzène sanguin et influence d'autres paramètres biologiques

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Introduction : L'acide *t,t*-muconique (MA) est actuellement considéré comme l'un des meilleurs biomarqueurs d'exposition au benzène. Cependant, des questionnements concernant sa spécificité (métabolite de l'acide sorbique, SA) et son intérêt pour évaluer de faibles niveaux d'exposition au benzène ont été soulevés récemment. Le but de cette étude était d'évaluer et de comparer l'utilité de différents biomarqueurs urinaires d'exposition chez des travailleurs de raffinerie exposés à de faibles niveaux de benzène (< 0,1 ppm).

Méthodes : 110 travailleurs (24 fumeurs ; 2 - 10 cigarettes/jour) ont accepté de participer à cette étude. Pour évaluer l'exposition interne, la concentration de benzène a été mesurée dans des échantillons de sang (B-B) prélevés en fin de poste de travail. Un échantillon urinaire a été récolté en fin de poste de travail en vue de la détermination du benzène non-métabolisé (B-U), de l'acide *S*-phénylmercapturique (SPMA), de l'acide *t,t*-muconique (MA), de l'acide sorbique (SA) et de la créatinine (créat.). B-U et B-B ont été déterminés par head-space/GC-MS (LQ : 0,10 µg/L), SPMA (LQ : 0,20 µg/L) et SA (LQ : 3 µg/L) par LC-MS, MA (LQ : 0,02 mg/L) par HPLC-UV et la créatinine par le réaction de Jaffé.

Résultats : La concentration médiane de B-B était 0,405 µg/L (de 0,10 à 13,58 µg/L). Les concentrations médianes des biomarqueurs urinaires étaient les suivantes : B-U 0,27 µg/L (de 0,10 à 5,35 µg/L), SPMA 1,40 µg/L (de 0,20 à 14,70 µg/L), MA 0,06 mg/L (de < 0,02 à 0,92 µg/L). Les concentrations de SA se situaient entre < 3 et 2211 µg/L (médiane 28,0 µg/L). Log B-B et log B-U, de même que log SPMA, contrairement à log MA, étaient

significativement plus élevés chez les fumeurs par rapport aux non-fumeurs. La meilleure corrélation entre le paramètre d'exposition interne (log B-B) et les biomarqueurs urinaires d'exposition a été obtenue avec log B-U (µg/L, $r = 0,514$, $p < 0,01$; µg/g créat., $r = 0,478$, $p < 0,01$) et log SPMA (µg/L, $r = 0,426$, $p < 0,01$; µg/g créat., $r = 0,495$, $p < 0,01$), suivi par log MA (mg/L, $r = 0,337$, $p < 0,01$; mg/g créat., $r = 0,313$, $p < 0,01$). Log SA et log MA étaient fortement corrélés ($r = 0,552$; après correction par la créat., $r = 0,557$). Une analyse de régression linéaire multiple a montré que log MA était principalement expliqué par log SA (30 % de la variance expliquée) et par log B-B (12 %). A ces faibles niveaux d'exposition, les polymorphismes génétiques des enzymes de biotransformation (CYP2E1, EPHX1, GSTM1, GSTT1, GSTP1) n'influençaient de manière significative les concentrations urinaires d'aucun des trois biomarqueurs.

Conclusion : Pour de faibles niveaux d'exposition au benzène, MA semble moins utile que SPMA ou B-U à cause de l'influence significative de SA provenant de l'alimentation. Par ailleurs, à ces niveaux d'exposition, l'influence des polymorphismes génétiques des enzymes de biotransformation sur la variabilité inter-individuelle de l'excrétion urinaire des biomarqueurs semble négligeable.

Mots clés : benzène, acide *t,t*-muconique, acide sorbique, acide *S*-phénylmercapturique

SOHT Meeting

22. Selective buprenorphine quantitation in blood and hair samples by LC-MS/MS detection of its N-methylpyridyl-derivatives

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Introduction: The potential of LC-MS in forensic toxicology is undisputed and includes substances of various pharmaceutical classes and chemical structures. Ionisation efficacy (e.g. electrospray ionisation) of toxicologically relevant compounds is mostly rather high due to the frequent presence of polar substituents. The comparatively low sensitivity of LC-MS detection of certain lipophilic compounds (e.g. steroids) lead to attempts to improve ionisation by chemical modification of the analytes. Derivatisation reactions using 2-hydrazino-1-methylpyridine (HMP), p-nitrobenzoyl chloride (NBC) and tris(2,4,6-trimethoxyphenyl)-phosphonium propylamine bromide (TMPP) may be applied to convert keto-, phenolic hydroxy- and carboxy- groups, respectively.

Methods: In the present study, the formation and LC-MS identification of buprenorphine-pyridyl-derivatives is described. Owing to several modifications of the morphinane structure (e.g. N-cyclopropylmethyl-substitution, ring closure by introduction of an 6 - 14 etheno-bridge) buprenorphine becomes rather unpolar and the molecule undergoes comparatively little fragmentation resulting in low product ion abundances in tandem mass spectrometry. In contrast, respective derivatives of buprenorphine form a series of selective and intense fragments.

Results: Detection limits and specificity of the buprenorphine derivatives were superior to those of unchanged buprenorphine under otherwise comparable LC–MS/MS conditions in serum and hair matrix. A detection limit of 0.1 ng/mL in serum (based on a sample volume of 500 µL) may be achieved. The stability of pyridyl derivatives was found to be sufficiently high in various solvents and permits to establish robust and reproducible buprenorphine quantitation procedures, e.g. in extracts from blood and hair samples.

Conclusion: Structural modification of substance properties by derivatisation –which is almost a default sample preparation in GC-MS– seem to have a considerable potential to enhance the sensitivity and specificity of LC-MS experiments in forensic toxicology.

23. Use of LC-MS/MS screening for hair analysis: application to detection of methadone in hair

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Introduction: Methadone is a widely used drug for the treatment of moderate to severe pain and for opioid dependency. This drug is widely abused largely by persons on heroin maintenance programs and is sometimes associated with administration to children for sedation. We present a series of cases in which methadone was detected in hair using LC-MS(MS).

Methods: For extraction of methadone, approximately 20 - 50 mg of washed hair was incubated with 2 mL methanol overnight at 60 ºC. The methanol was transferred into a glass tube and evaporated to dryness. The extracts were reconstituted in mobile phase and injected into a LC-MS/MS. The extracts were separated on an Agilent Eclipse XBD C18 (4.6x150 mm, 5 µm particle size) using ammonium formate (pH 3.5, 50 mM) buffer and 1% formic acid in acetonitrile and gradient analysis. Methadone and other drugs of abuse were detected using an Applied Biosystems 3200 Q-Trap mass spectrometer coupled with a Turbo Ion Spray source and operated in MRM mode using 3 transitions per analyte. Methadone was detected using the following transitions: 310→265; 310→105, and 310→77; for detection of EDDP the transitions 278→234, 278→249, and 278→186 were used. Alternatively extracts were also analyzed conventionally by GC-MS using a model 5972 Agilent system on a BP-5 capillary column.

Results: The method gave reproducible and sensitive detection for methadone and 29 other common drugs or drug metabolites**.** The LC-MS/MS method was validated for all substances in hair. The limits of quantification for methadone and EDDP were 0.1 ng/mg of hair, based on the lowest calibrator. Limits of detection for methadone and its main metabolite were below 0.01ng/mg hair, determined using spiked hair samples. The precision for methadone and 29 other common drugs or drug metabolites at 0.25 and 25 ng/mg was found to be within 10% coefficient of variation, accuracy was > 90%. Four cases involving children suspected of unprescribed exposure to methadone showed the presence of the drug in concentrations ranging from the LOQ to 3 ng/mg, with an average of 1.2 ng/mg. In two cases segmental analyses showed presence of methadone in a number of 1 or 2 cm segments. In another two cases multiple segments were also positive. In some segments the metabolite (EDDP) was also detected in much lower concentrations. Other drugs were also detected in some of the cases suggesting multiple drug exposure. This presentation will provide details of the cases together with analytical performance data to support the use of LC-MS(MS) for the detection of drugs in hair extracts.

Conclusion: Hair analyses for methadone can provide some useful information on a case, however interpretation can be complex.

24. Detection of benzylpiperazine (BZP) in hair as part of a quantitative multi analyte drug screen using LC-MS/MS

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Introduction: Benzylpiperazine (BZP) is an amphetamine type stimulant, currently legally available in New Zealand and widely used in 'Party Pills'. Following consultation with an expert scientific committee, the NZ government proposed a reclassification to Schedule C (the same as cannabis), due to come into force at the end of January 2008. Many methods have been published for the analysis of drugs of abuse in hair, for this method we have included BZP with amphetamine type stimulants, opiate type drugs and methadone in a single assay.

Method: Hair samples are segmented into the growth periods of interest and cut finely before weighing. Each analysis needs one 20 mg sample to test for: amphetamine type stimulants (BZP, amphetamine, methamphetamine, methylenedioxymethamphetamine and methylenedioxyamphetamine);

opiate type drugs (codeine, morphine and 6 acetyl morphine); and methadone. Internal standard (deuterated analogue of each drug) was added to each sample. A five point calibration curve was prepared for each analyte. Following soaking in HCl overnight the extract was applied to a solid phase extraction cartridge (Bond Elut Certify) and eluted using dichloromethane: propan -2-ol: ammonium hydroxide (80:20:2). The sample is then dried before reconstitution (in 100 µL of mobile phase) for analysis by LC-MS/MS. A single injection of 10 µL is applied to a Phenomenx Luna SCX column (150 x 2.0 mm) using isocratic elution with acetonitrile (75%) and ammonium formate buffer (100 mM) +0.5% formic acid (25%) as mobile phase. This assay has been used in various scenarios, such as investigation in drug facilitated sexual assault or in child custody cases.

Results: Linear calibration (<0.999) was seen for the range 0.2 to 50 ng/mg of hair analysed (based on 20 mg). Spiked samples of 1.25 ng/mg gave intra- and inter-day precision CVs of 5% and 8% respectively (n=6 per day, 3 days). Extraction efficiency was calculated to be 68% and accuracy was 108%. Results for all analytes were acceptable.

Conclusion: This paper presents a fully validated assay for the analysis of multiple classes of drugs from a single hair extract using a single LC-MS/MS injection. The use of the assay will increase when the legislation changes and BZP becomes controlled under law.

Keywords: hair, benzylpiperazine, LC-MSMS

25. Monitoring methylphenidate treatment in children and adolescents by hair and saliva testing

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Introduction: Methylphenidate (MPH) is a phenethylamine derivative used in the treatment of attention-deficit hyperactivity disorder in children and adolescents. It is known that there is marked individual variability in the dose-response relationship for methylphenidate, and therefore dosage must be titrated for optimal effect and avoidance of toxicity in each child. Therapeutic monitoring for this drug is essentially lacking and alternative biological matrices should be investigated for non-invasive assessment of short and long term recond of drug use. We sought to monitor MPH treatment in children and adolescents by hair and saliva testing.

Methods: Hair samples were obtained from 35 subjects, diagnosed for ADHD and in treatment for at least the last six months with different oral doses of immediate and controlled-release MHP (from 5 mg to 36 mg/day). Whereas possible, (hair shaft length more than 3 cm) segmental hair analysis was performed of subsequent 3 cm hair strands, each representing hair growth in subsequent three-month periods. Saliva samples were obtained from 4 subjects starting treatment with 20 mg controlled release MHP. Samples were collected to investigate the 24 hour kinetics once a week. Using 3,4-methylenedioxypropylamphetamine as internal standard, hair samples were overnight digested with 0.1M HCl at 37°X and MHP extracted with Bond-Elut Certify columns while saliva samples (500 µL) were added with 500 µL of acetonitrile, mixed, centrifuged and organic phase evaporated to dryness. A procedure based on liquid chromatography-mass spectrometry (LC-MS) was applied, using a reverse phase column and a mobile phase of 80% 10 mM ammonium acetate – 20% acetonitrile with a 15 min gradient program and the mass spectrometer in positive electrospray ionization and selected ion monitoring acquisition mode. The method was validated in the range 0.15 - 50 ng MPH/mg hair and 0.15 - 50 ng MHP/mL saliva.

Results: Preliminary results on hair segments showed that, even in presence high inter-individual variability in hair segments concentration of MPH for children treated with the same dose, the mean values of MHP (from 0.48 to 1.29 ng MHP/mg hair) in different hair sections well correlated (r^2=0.7)with drug dosage (from 10 to 36 mg/day). Analysis of salivary samples gave 24 kinetic profiles of 20 mg controlled release MHP with a steady state mean concentrations ranging from 10.61 ng/mL to 1.65 ng/mL one to 9 hours after drug administration, respectively. At 12 hours concentrations decreased to a mean value of 0.16 ng/mL saliva.

Conclusion: These data indicate the possible use of segmental hair analysis to monitor compliance of subjects under long-term treatment with MHP and the use of saliva as alternative to blood for MHP monitoring and consequent dosage titration once therapeutic and toxic concentration ranges will be established in this biological matrix.

Keywords: methylphenidate, hair testing, saliva testing

26. Development and application of reference materials for the determination of methamphetamine and amphetamine in hair

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Introduction: Methamphetamine (MA) has received the most attention as a drug of abuse in Korea. Thus, hair analysis for MA is critical because it is accepted by law enforcement agencies as one of important corroborative facts for MA abuse. As part of quality control, the need for a reference material (RM) for hair analysis has rapidly increased in our laboratory. In the present study, we developed two RMs, NISI RM 0711-01 and 0711-02 using authentic and drug-free hair samples, respectively, according to the recommendations of ISO Guide 35 for the determination of methamphetamine and its main metabolite, amphetamine (AP) in human hair. Moreover, the prepared RMs were distributed to eight participants in four laboratories under the National Institute of Scientific Investigation in Korea for the purpose of internal quality control.

Methods: For the preparation of NISI RM 0711-01 (103 vials, ca. 100 mg each), MA abusers' hair samples were collected, homogenized and finally bottled. For the preparation of NISI RM 0711-02 (97 vials, ca. 100 mg each), drug-free hair was soaked into the DMSO solution containing MA and AP until the concentrations of MA and AP were plateaued. The concentration of each bottle was determined using two extraction methods, agitation with 1% HCl in methanol at 38°C and ultrasonication with methanol/5M HCl (20:1), followed by gas chromatography/mass spectrometry (GC/MS) after derivatization with trifluoroacetic anhydride (TFAA). The homogeneity of analytes was evaluated and their property values were determined with their uncertainties. Also, statistical analysis was conducted with the results of the internal proficiency test, where average, median, normalized inter quartile range (NIQR), Robust CV and Robust Z score were calculated.

Results: Satisfying homogeneity was reached for MA and AP in the prepared two RMs. Finally, the certified values of NISI RM 0711-01 were 7.64 ng/mg and 0.54 ng/mg and their expanded uncertainties were 1.05 ng/mg and 0.07 ng/mg for MA and AP, respectively. NISI RM 0711-02 was prepared at the level of 4.86±0.55 ng/mg and 4.63±0.44 ng/mg for MA and AP, for each. In the internal proficiency test, most participants showed satisfying performances except one with NISI RM 0711-01 and one with NISI RM 0711-02, where corrective action should be undertaken.

Conclusion: The preparation and/or use of RMs as well as the management and/or participation of proficiency tests are main areas in the quality assurance of analytical chemistry laboratories. Especially, the RM is indispensable to assess the trueness and precision of their measurement methods. The RMs we developed here can be useful in forensic laboratories for internal quality control and external quality assurance.

Keywords: hair analysis, reference material, proficiency test

27. Hair extraction recoveries from intact and powdered hair into methanol

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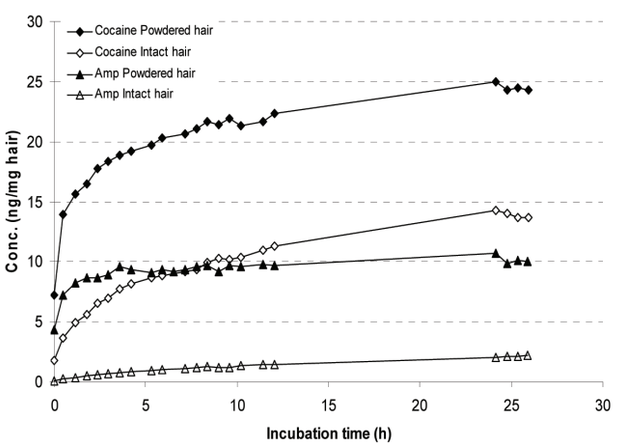
Introduction: Reports on drug detection in hair based on incubation of the hair in organic solvents or solvent buffer mixtures without the disintegration of hair are common. We have previously shown variations in extraction kinetics from hair into aqueous media. Obvious risks with such procedures are less than 100% recovery owing to a short extraction time, morphological differences in hair samples or degradation of analytes. The aim of this study was to compare the extraction recoveries of cocaine, bensoylecgonine, codeine, amphetamine and MDMA into methanol from intact or powdered hair.

Methods: Two incubation procedures were compared, one using a shaking water bath and a procedure using sonication. The experiments were performed on a pool of authentic hair. Prior to the extraction, hair samples were washed thoroughly with our standard protocol including an initial wash with iso-propanol, and then three washes with phosphate buffer and a final iso-propanol wash. Five replicates of each experiment were performed. The stability of the analytes during incubation was verified in separate experiments. To 10 mg of hair was then added 2.0 mL of methanol and 50 µl of internal standard and the sample was incubated in a water bath (with orbital shaking) at 37 ºC for 26 hours or in an ultrasonic bath at 50 ºC for 12 hours. At 30 minute intervals a 20 µL aliquot was removed from the incubation mixture, diluted 1:2 with water and 1 µL was injected into the LC-MS-MS system. The LC-MS-MS analysis was performed on a SCIEX API 4000 MS-MS instrument equipped with an electrospray interface.

Results: Comparing the 12 h fractions, sonication did not show higher recoveries than incubation in a normal water bath. However, powdered hair generally showed much more rapid kinetics initially, followed by a slower increase or a plateau for the remaining incubation. For all analytes except bensoylecgonine, the powdering of hair markedly increased the recovery, however the impact was different depending on the drug as depicted in the figure. For amphetamine and codeine, the recovery was approximately five times higher using powdered hair whereas for cocaine and MDMA it was twice as high. Different extraction recoveries for cocaine and bensoylecgonine from powdered and intact hair resulted in different metabolite/parent compound ratios. Using powdered hair, the ratio became 2-3 times lower because of the higher recovery of cocaine.

Conclusion: We conclude that sonication did not improve recovery. The procedure with powdered hair gave higher recoveries but the increase was substance dependent.

Again, this emphasizes that each laboratory evaluate their methods and criteria for a positive result.



28. Extraction of drugs of abuse from hair. Can we determine realistic extraction recovery?

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Introduction: Drug analysis in hair has become very popular in recent years due to many advantages over 'traditional' matrices. Being able to test the hair for drug exposure long after the drug has been consumed is the principal reason for performing hair analysis. Drugs of abuse are incorporated into hair and many analytical applications have been published over the last decades. Quantitative methods for the detection of drugs of abuse in hair are usually validated using spiked hair samples. A complete digestion of the hair matrix is problematic as many drugs of abuse (e.g. heroin, cocaine, benzodiazepines) are not stable under the conditions of digestion. Methanolic incubation has become the most common extraction method for drugs of abuse from hair. Extraction recovery determination using spiked hair samples may not give accurate results as the analytes in spiked hair samples are external of the hair, and not contained within the hair. Therefore, the aim of the study was to compare extraction recoveries of spiked hair samples, fortified hair samples and real hair samples using multiple extractions.

Methods: For extraction of drugs of abuse, 100 mg of hair was incubated with 2 mL methanol overnight at 60ºC. The methanol was transferred into a glass tube and evaporated to dryness. The extracts were reconstituted in mobile phase and injected into LC-MS/MS. To the hair samples, 2 mL of methanol was added, extracted and analyzed as described above. This extraction and analysis was repeated at least five times (exhaustive extraction). The following hair samples were used for analysis: Five different blank hair samples spiked with 30 common drugs of abuse, five different blank hair samples fortified with 30 common drugs of abuse and five different hair samples positive for common drugs of abuse. The extracts were separated on a Hypersil C18 column using gradient analysis. The drugs of abuse were detected using an Applied Biosystems 3200 Q-Trap mass spectrometer operated in MRM mode.

Results: Spiked hair showed for most drugs of abuse recoveries of ~90% following single extraction; by the third extraction only trace amounts of drugs could be detected in spiked hair samples. In fortified and real hair samples, extraction recoveries in the first extraction step varied significantly between 20 and 80%, but most drugs were still detectable in the fourth and fifth extractions. For example the drug tramadol showed recoveries of 90%, 98%, 100% in spiked hair following 3 consecutive extractions. No drug was detected in further extractions. In contrast, the fortified hair sample, tramadol showed recoveries of 71%, 92%, 98%, 100% following consecutive extractions and in real hair; the recoveries were 72%, 90%, 96%, 100%. To further complicate the range of recoveries determined identical fortified hair samples also gave significantly different results. Amphetamine for example was extracted from a fortified hair sample with recoveries of 55%, 84%, 97%, 100% after consecutive extractions, whereas an identical hair sample under identical extraction conditions showed recoveries of 86%, 95%, 99%, 100%.

Conclusion: Spiked hair samples showed significant differences from fortified and real hair samples in terms of extraction recoveries after multiple extractions. Using spiked hair samples for calibration may lead to miscalculated concentrations in real hair samples. It is also clear that by performing 3 or more extractions, maximum recovery of drug can be achieved from either fortified or real hair specimens.

29. Testing for alcohol use in hair: is ethyl glucuronide (EtG) stable in hair?

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Introduction: The usefulness of biological markers of alcohol misuse, such as the measurement of ethyl glucuronide (EtG) in hair, is still under evaluation. There is a view that when EtG is detected above certain levels, it is strong evidence of moderate to excessive alcohol use; and that negative results are indicative of abstinence. However, there is also a consensus that positive results should not be regarded as definitive, but corroborative evidence. A negative result is also not definitive of abstinence because as it is not possible to rule out the effects of normal hygiene practices in hair such as shampooing, which will affect retrospective estimation of alcohol consumption over a period of many months. The main aim of this paper is to open the discussions on the usefulness of sectioning analysis of head hair samples in view of the potential limitations and to aid in the discussions of the subject.

Methods: 181 hair samples were tested for EtG, 102 of those were analysed as multiple sections of one month, covering three months, the first section being the most recent period and the third section the earliest period. The hair sections were washed with methanol dried and submitted to overnight sonication in water. The samples underwent SPE using anion exchange cartridges, followed by derivatisation with BSTFA before analysed by GC-MS/MS. The assay was linear over the calibration range 0.01 ng/mg–0.5 ng/mg, and analytical cut-off was 0.010 ng/mg of hair assuming a 20 mg hair sample.

Results: The 95% percentile of the EtG levels detected in the first section for all the samples and for the group of samples analysed as multiple sections, were 0.21 ng/mg (N=181) and 0.22 ng/mg (N=102), respectively. The 95% percentiles of the levels detected in the second section and in the third section were 0.15 ng/mg (N=102) and 0.10 ng/mg (N=87), respectively. Of the samples where multiple sections were analysed, 67% (N=68) showed levels of EtG below the cut-off in all sections. Of the samples where EtG was detected, 65% of the second section samples (N=22) showed EtG levels on average 50% lower the levels detected in the first section. The levels detected of EtG in month three were on average 45% the levels of the previous month and 71% the levels detected in the first month. Analysis of variance showed the levels of the third section significantly lower ($p<0.05$) than the first section. The trend for the levels of the most recent hair growth being considerably higher than the preceding months could be due to normal hair hygiene, as EtG is soluble in water, and not due to any pattern of alcohol use.

Conclusions: The results of this study suggest that normal hair hygiene might wash out EtG from the hair producing the trend seen in the group of samples studied above. EtG in hair can be very useful diagnostic instrument for alcohol dependency, but in isolation could be misleading, even though the laboratory results are accurate. Unlike drug testing in hair of illicit drugs, where a single positive test can be sufficient to conclude that a person used drugs, a positive, EtG result can be only 'suggestive' of alcohol misuse or a negative result 'indicative' of abstinence and should not be regarded as definitive. We still need to be cautious regarding the interpretation of the results until further scientific evidence regarding the stability of EtG in hair becomes available. The recommendation is therefore that only the most recent month be tested for alcohol use using head hair and that data should be evaluated in conjunction with other biochemical tests and clinical evaluation.

30. Ethylglucuronide as a biomarker of ethanol intake: Application to clinical and forensic cases

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Introduction: The ethyl glucuronide (EtG) is a specific biomarker for ethyl alcohol intake that has some advantages with respect to other biomarkers. In recent years there have been several papers published in relation to its forensic and clinical applications. The aim of this work has been to validate a LC-MS/MS analytical method in clinical and forensic samples (blood, urine, hair and vitreous humour), as well as its application to real cases in patients following Alcohol Dependence Programs (ADP), and corpses which have undergone legal autopsies.

Methods: Urine (n=568) and hair (n=23) samples were taken from patients following ADP. The autopsy samples (n=71) were taken from recently deceased bodies (postmortem interval <24 hours) with no ocular damage. Ethanol levels were calculated by headspace gas chromatography (HS-GC), equipped with a Flame Ionization Detector (FID), with a limit of detection of 0,05 g/L. EtG levels were analyzed by Liquid Chromatography Mass Spectrometry in ESI conditions and using D5 EtG as Internal Standard. MS experiments were performed in Multiple Reaction Monitoring (MRM) following these transitions: EtG 221,2>74,7, 221,2>84,8, 221,2>221,2; EtG d5: 226,2>74,7, 226,2>226,2.

Results: The methods were fully validated in all samples with the following detection limits (LOD): 0,1ug/mL for urine, 0,025 µg/mL for blood and vitreous humour and 0,025ng/mg for hair. Quantitation limits (LOQ) were 0,25 µg/mL, 0,05 µg/mL and 0,05 ng/mg, respectively. Inter day and intraday CV were lower than 20%, no interfering peaks in any blank sample**.** From the analysis of 568 urine samples, 124 were positive for EtG (range: 0,1-785 µg/mL, mean 46,5 µg/mL), while only 19 were positive for Ethanol. All the positive cases for ethanol were positive for EtG. Of the 23 hair samples only 8 were positive for EtG (range: 0,09 - 0,64 ng/mg). Of the 71 forensic samples 21 were positive for ethanol in vitreous humour and of these 16 were also positive for EtG (range=0,03 - 2,6 µg/mL).

Conclusions: The application of LC-MS/MS in the detection of EtG is an efficient analytical procedure for clinical and forensic purposes. In clinical cases, urine samples can provide immediate information about the patient's progress. EtG can be detected in vitreous humour but in very low concentrations and to understand the meaning of these levels, a detailed kinetic study of this compound is needed.

31. Determination of ethyl glucuronide in hair by derivative HS-SPME/GC-NCI -MS and methodical comparison with liquid injection-GC-NCI-MS and with LC-MS/MS

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Introduction: Alcohol markers in hair (ethyl glucuronide EtG and fatty acid ethyl esters FAEE) have found increasing interest for retrospective detection or exclusion of alcohol abuse. The concentrations of EtG found in cases of chronic excessive drinking vary over a large range from some pg/mg to 10 ng/mg and, according to the present state of experience, 7 pg/mg and 25 pg/mg are applied as the upper limits for abstinence and social drinking respectively. Sensitive routine techniques are required for accurate determination of EtG at these low concentrations. Therefore, a method based

on derivative headspace solid phase microextraction (HS-SPME) and gas chromatography - negative chemical ionization mass spectrometry (GC-NCI/MS) was developed and applied to hair samples of teetotalers, social drinkers and alcohol abusers. The method is compared with liquid injection-GC-NCI/MS and with LC-MS/MS.

Methods: For all three methods, the hair samples were subsequently washed with water and acetone, and dried. About 30 mg were extracted with water and the aqueous extract was cleaned-up by solid phase extraction using Oasis® Max 3 ccm anion exchange columns (Waters Inc.). D_5-EtG was used as the internal standard. For determination by HS-SPME, the eluate (2% HCOOH/methanol) was evaporated, derivatized with heptafluorobutyric anhydride (HBFA) and again evaporated to dryness. The dry residue was submitted to HS-SPME without any additional agents. The method was optimized with respect to the SPME fiber (75 µm Carboxen/PDMS) as well as temperature and time of derivatization (30 min at 80°C) and extraction (22 min at 105°C). The m/z 397, 399 and 596 for EtG and 402, 404 and 601 for D_5-EtG were used for GC-MS-NCI-SIM. For direct injection GC-NCI-MS the residue of the eluate was derivatized with pentafluoropropionic anhydride and measured as described previously. In case of LC-MS/MS, the residue was dissolved in 60 µl of the mobile phase (0.1% HCOOH in H_2O/Acetonitril 93:7 v/v) and 50 µl were injected with the use of a 10 x 2 mm 5 µm Hypercarb HPLC column. For comparison, all three methods were applied to a series of hair samples.

Results: In the evaluation of the automated HS-SPME method, a limit of detection (LOD) of 4 pg/mg and a limit of quantification (LOQ) of 9 pg/mg were obtained. In application to real samples the concentrations were reproducible. The results from the three methods, which were performed in different laboratories, were qualitatively in a good agreement, i.e., no false positive or false negative results were obtained with respect to the cut-offs given above. Quantitative differences can be explained by differences in sample preparation, extraction procedure and inhomogeneities of the samples.

Conclusion: It follows from this investigation that HS-SPME in combination with derivatization and GC-MS can be applied for polar substances such as EtG. The headspace extraction from the dry residue is an additional clean-up step and delivers reproducible results if deuterated standards are used. All three methods are suitable for practical application. It is seen from the methodical comparison that the most serious reason for uncertainty is the sample preparation and not the measurement.

Keywords: ethyl glucuronide, hair, HS-SPME/GC-NCI/MS

32. 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid ethyl ester (THC-COOEt): Unsuccessful search for a marker of combined cannabis and alcohol consumption

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Introduction: In analogy to cocaethylene, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid ethyl ester (THC-COOEt) can be presumed to be a mixed metabolite formed during combined consumption of cannabinoids and alcohol. This hypothesis was studied by investigation of blood and hair samples of cases with known cannabis and alcohol use.

Methods: THC-COOEt and its deuterated analogue D_3-THC-COOEt were synthesized as reference substance and internal standard from the corresponding carboxylic acids and diazoethane and identified by GC-MS. For determination in blood (serum, plasma), solid phase extraction and subsequent derivatization with N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide (MBDSTFA) in case of GC-EI-MS and with pentafluoropropionic anhydride (PFPA) in case of GC-NCI-MS were used. Hair samples were analyzed by extraction with a two-phase mixture of aqueous buffer (pH 7.6) and iso-octane in ultrasonic bath for 15 h, separation and evaporation of the organic phase, derivative headspace solid-phase microextraction (HS-SPME) in presence of MBDSTFA and measurement by GC-EI-MS. For increase of the sensitivity, the derivatization was performed with PFPA followed by measurement using HS-SPME combined with GC-NCI-MS and GC-NCI-MS/MS.

The methods were applied to plasma samples from 18 drunk driving cases and four other volunteers which contained both ethanol (0.30 to 2.16 mg/mL in whole blood) and THC-COOH (7.6 to 252 ng/mL in plasma) as well as to 15 hair samples from drug fatalities or volunteers which were both positive for THC (0.05 - 2.04 ng/mg) and fatty acid ethyl esters as markers of chronic alcohol abuse (0.2 - 6.3 ng/mg).

Results: In none of these samples THC-COOEt could be found with limits of detection of 0.3 ng/mL in plasma and 0.01 pg/mg in hair.

Conclusion: Different from the formation of cocaethylene or fatty acid ethyl esters, there seems to be no efficient way of the metabolic formation of THC-COOEt. As a reason, the missing biochemical activation of the carboxylic group is discussed. Therefore, a use of this compound as a marker for combined cannabis and alcohol consumption appears not to be possible.

Keywords: hair analysis, marker of combined alcohol and cannabis consumption, 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid ethyl ester

33. High prevalence of positive FAEE hair tests among families involved with children's aid societies and associated concurrent drug use

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Introduction: The FAEE hair test is a novel biomarker used to assess excessive chronic alcohol consumption. The test holds significant potential for facilitating diagnosis of Fetal Alcohol Spectrum Disorders that are associated with heavy maternal alcohol consumption during pregnancy. Never before has the use of the FAEE hair test been reported in the context of assessing parental alcohol use. The current study aims to evaluate the prevalence of heavy alcohol use in a cohort of caregivers involved with children's aid societies, and its relation to concurrent drug use as measured by hair analysis.

Methods: A cohort study was performed and included all parental hair samples sent by children's aid organizations to the Motherisk Laboratory in Toronto, Canada, for FAEE analysis between October 2005 and December 2008. FAEE (ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate) were analyzed according to a previously established protocol. Briefly, approximately 20 mg of hair was weighed and chopped into 1 - 3 mm segments. FAEE were extracted using a liquid-liquid extraction involving n-heptane and dimethyl sulfoxide, and analyzed using head space solid phase microextraction (HS-SPME) coupled with gas-chromatography (GCMS). HS-SPME conditions were as follows: samples were preheated for 5 minutes at 90°C, 250 rpm agitation, then absorbed over 30 minutes at 90°C, 150 rpm agitation, then desorbed for 15 minutes at 260°C. The agitation mode was 60s right, 30s interval, 60 s left, 30 s interval, etc. GCMS injector, interface, ion source and quadrapole were at 260°C, 310°C, 230°C, and 70°C, respectively. The temperature program was 2 min at 70°C, then 20°C/min up to 300°C, hold 0.5 min at 300°C. The LOD and LOQ values were previously reported to be between 0.01 ng/mg for ethyl sterate and 0.04 ng/mg for ethyl oleate for LOD, and between 0.04 ng/mg and 0.12 ng/mg for corresponding LOQ values. Odds ratio analysis was conducted on all samples that were also concurrently tested for one or more drugs (amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, methadone, oxicodone), in order to evaluate associations between heavy alcohol use and drug use.

Results: A total of 346 samples were tested for FAEE and 33% yielded positive results above 0.5 ng FAEE/ mg hair, marking heavy chronic alcohol consumption. Significant odds ratios between testing positive for FAEE and methamphetamine (OR=4.50, 1.10 - 18.34), and cocaine's metabolite benzoylecognine (OR=1.84, 1.02 - 3.34), were found.

Conclusion: The current investigation reports for the first time ever the level of positivity for heavy alcohol use as measured by the FAEE hair test in a cohort of parents involved with children's aid services. The high prevalence of positive FAEE tests, one third of the cohort, demonstrates the utility of and need for this biomarker as a tool in the child welfare community as well as the hair tests' potential as a screening tool for maternal alcohol use associated with the pathogenesis of Fetal Alcohol Spectrum Disorders. The study also confirms the known association between alcohol and drug use, but for the first time ever measures this relationship using hair analysis, demonstrating the validity and usefulness of this technique.

34. Control of abstinence or proof of consumption: hair analysis as a tool within the process of re-granting the driving licence

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Introduction: Testing the driving ability for re-granting the driving licence is a major task of the department of traffic medicine of our institute. Driving ability can be defined as a general, not time restricted or case dependent, psychological and physical capability to drive a car safely. These examinations are required by the driving licence authorities in cases of driving under influence of drugs and/or alcohol. The driving licence is only re-granted if a drug or alcohol abuse can be excluded. Very often, drug abstinence or teetotalism is to complied with for a certain period of time after re-granting the licence.

Results: In cases of alcohol and/or drug abuse, hair analysis is a powerful tool in the examination to re-grant the driving licence. In our lab, the number of hair analyses of drugs of abuse increased from 60 cases in 2003 to about 850 cases in 2007. Since 2006 we also use Ethylglucuronid (EtG) determination in hair (about 300 hair analysis in 2007) to check for teetotalism or to detect repeated excessive alcohol consumption, respectively. During these last years, different studies were performed in our institute. Standards of the examination of the driving ability were expressed as outcome of a study with Cocain users: 65% positive hair samples of test persons with negative tested urine samples (immunoassay test) during a six months period before taking the hair sample, and 66% positive hair samples of subject who stated explicitly that they never have consumed Cocain. In a study with 154 cases EtG was analyzed in the hair samples and CDT-values were determined either by an immunoassay test or by HPLC. 84 cases were positive for EtG, thereof 39 cases with CDT-values above normal range and 45 with CDT in a normal range (<2,6%, immunoassay test), and 15 cases with CDT-values above normal range and 69 with CDT in a normal range (<1,77%, HPLC), respectively. Our guidelines and the quality management will be outlined.

Conclusions: Actually, there is no uniform examination procedure to re-grant the driving licence in the different cantons of Switzerland. In case of a licence withdrawal due to an abuse of drugs and/or alcohol, we strongly recommend a hair analysis as part of the examination. This tendency can also be observed in other European countries. At present, we are completing our protocol for the hair analysis procedure. Hair analysis will be used firstly to proof or exclude drug or alcohol abuse during the driving ability assessment and secondly - after the driving licence is re-granted - to supervise the drug abstinence and/or the teetotalism in the following 6 to 12 months. Our proposals have been adapted to the official recommendations. Different decisions of the federal court of Switzerland and of some cantonal courts have affirmed that a positive result of a hair analysis is a proof of consumption of alcohol or drugs within a certain period of time.

Keywords: hair analysis; driving ability; re-granting the driving licence, guidelines; quality management

35. Simultaneous determination of 18 benzodiazepines and their main metabolites in hair, blood and urine by LC-ESI-MS/MS. Application to the determination of triazolam in a drug-facilitated crime

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Introduction: We present an application of liquid chromatography- triple stage quadrupole-tandem mass spectrometry with electrospray probe (LC-ESI-MS/MS) for the determination of traces of 18 benzodiazepines (BZDs) and their main metabolites in biological specimens (hair, urine and blood). This technique is at this time one of the most powerful analytical methods to be used for qualitative and quantitative analysis of drugs traces in blood, urine and hair. Furthermore, segmental hair analysis provides retrospective information helpful to solve drug facilitated crimes in forensic expertise. A case report with triazolam is presented.

Methods: To 20 mg of decontaminated and cut hair, 100 pg/mg of clonazepam-d4 (Cerilliant provided by Promochem), was added as internal standard. Hair specimens were extracted with 2 mL dichloromethane/ ether (80/20) after incubation one night at 56°C in Soerensen buffer pH 7.6. After centrifugation, the organic layer was filtered with PTFE 0.2 µm then evaporated to dryness at ambient temperature. Urine and blood were extracted with Toxi-tube A® (Varian) and with 5 ng/mL of clonazepam-d4. The residues were reconstituted by 100 µL of MeOH/ACN/ Formate buffer (25/25/50) and transferred in glass vials. Ten microliters were injected into the LC- MS/MS TSQ Quantum Ultra (ThermoFisher). Separation was achieved on a C_{18}-column (Uptisphere ODB 150 x 2 mm–5 µm) at 30°C. Mobile phase (formate buffer 2mM pH 3 / ACN) was delivered in gradient mode for a total run time of 17 min. The mass spectrometer was operated in selected-ion monitoring mode with fragmentation of [M+H]+ ions. To each pseudo-molecular ion 2 to 3 product ions were acquired at a scan time of 0.1 s and a width of 1.0 a.m.u.

Results: Standard curves in hair (0.5 - 100 pg/mg) were prepared by spiking aliquots of blank hair and had r^2>0.9877 for all BZDs. LOD ranged from 0.5 - 2 pg/mg. Standard curves in blood and urine (0.5 - 100 ng/mL) were prepared by spiking aliquots of blank fluids and had r^2> 0.9816 for all BZDs. LOD ranged from 0.5 - 1 ng/mL.

We applied this method to the determination of benzodiazepines and analogues in blood, urine and hair of a 58-year-old Japanese lady after she was robbed at home following the ingestion of a suspect coffee brought from a fast food by a compatriot. She awoke about twenty four hours after the drunk the coffee. Biological fluids were sampled 61 hours after the offence and hair was collected 15 days later. Analysis of blood showed no traces of triazolam (mean t1/2: 1.5 - 3h, therapeutic range: 2–20 ng/mL) while hydroxy-triazolam was determined in urine at a concentration of 2.6 ng/mL after hydrolysis. Three segments (2-cm) from the root of hair were analyzed. Triazolam was detected only in the proximal segment at a concentration of 1.3 pg/mg (LOQ: 1 pg/mg).

Conclusion: The low concentration of hydroxy-triazolam in urine 61 h after the offence and the low concentration of triazolam in hair, only in the proximal segment, were in accordance with a single intake. These results show the usefulness of LC-MS/MS as well as segmental hair analysis for the elucidation of drug facilitated offences or crimes. To our knowledge, it is the first report of the determination of triazolam in human hair after single intake.

Keywords: benzodiazepines, triazolam, drug-facilitated crime, LC-MS/MS

36. Midazolam drug-facilitated crimes: three recent observations in hair

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Introduction: We report 3 drug-facilitated crimes in which midazolam, an exclusively hospitable benzodiazepine in France was used. Cases 1 and 2: Two young women in their twenties became cocaine-addicted under the bad influence of the same man. They underwent rapes and mutilations. Those brutalities lasted months, or even years. These two women mentioned a ketamine intake without their knowing, the association cocaine/ketamine allowing them not to feel pain. This man is presently prosecuted for narcotics detention, breach of weakness and harmful substances administration. Case 3: A 73-years-old American tourist invited a male in his hotel room for a drink. He was found 3 days later in a comatose state and with a head wound. All his valuables (money, jewellery…) had been stolen. He has been hospitalised for 5 days after his aggression. The suspect is sued for robbery and rape.

Method: Each hair strand is divided in 1 cm-long segments corresponding at a period of about 1 month. A specific liquid-liquid extraction is realised for each molecules class (cannabinoids, opiates and cocaine, benzodiazepines, hypnotics, anaesthetics). Narcotics are detected by GC-MS and/or GC-MS/MS. 26 benzodiazepines and metabolites, and 12 hypnotics or sedatives are analysed by GC-MS, GC-MS/MS and/or HPLC-MS/MS. GHB is quantified by GC-MS. The whole hair strands of cases 1 and 2 are analysed (between 23 and 31 segments) because the facts lasted over a long period. Facts of case 3 happened only one month before the hair sampling, that's why only 3 segments are analysed.

Results: Case 1: Cocaine (9 - 178 ng/mg) and its metabolites, as well as MDMA (0.7 - 36.3 ng/mg) and its metabolite MDA were found overall the hair strand indicating a chronic intake of these drugs. The hypnotic zolpidem (20 - 280 pg/mg) was present over two 6-months long periods. The victim took regularly Stilnox®. Diazepam (80 - 90 ng/mg) and its metabolites were detected in three distinct segments and might have been prescribed to the victim to treat her depressive moods. Finally, midazolam (50 - 200 pg/mg) was identified in three different segments that might fit with the facts period. Its effects are similar to those of ketamine, anaesthetic absent of the victim's hair. Case 2: As previously, cocaine and its metabolites were detected overall the hair strand. The victim declared to have taken the following detected drugs: bromazepam (0.1 - 1.2 ng/mg), clonazepam (0.2 - 117.8 ng/mg), cyamemazine (0.4 - 14.6 ng/mg) and alimemazine (0.1 - 2.7 ng/mg). Zolpidem (0.1 - 2.4 ng/mg) was detected in all segments. Zopiclone (0.2 - 2.5 ng/mg) and lorazepam (2.2 - 33.3 ng/mg) were identified over a long period and nordazepam (270 pg/mg) in one segment; they can fit with treatments prescribed to the victim for drug addiction during hospitalisation. As in case 1, midazolam (130 - 170 pg/mg) was detected in three distinct segments whereas no ketamine was revealed. In conclusion, cases 1 and 2 put in light two chronic poly-intoxications: results are in agreement with the victims statements about the drugs they took. The other drugs are concordant with different cures the victims followed. Only presence of midazolam is surprising but this compound can have been mistaken with ketamine. Case 3: Hair analysis showed the presence of only one molecule in the segment corresponding to the facts: midazolam (150 pg/mg).

Conclusion: Although midazolam is not the most frequently found compound in drug-facilitated crimes and is quite difficult to get because of its exclusively hospitable use in France, it is nevertheless indispensable to include systematically its research in the analysis. The three cases described above took place in very different drugs contexts (drug naïve or excessive consumer) and give concentrations in hair that complete the few existing data.

Keywords: midazolam, drug-facilitated crimes, hair

37. Age and chemical abuse. Evidence from hair analysis

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Introduction: The use of a drug to modify a person's behaviour for criminal gain is not a recent phenomenon. However, the recent increase in reports of drug-facilitated crimes (sexual assault, robbery) has caused some alarm in the general public. Drugs involved can be pharmaceuticals, such as benzodiazepines (flunitrazepam, lorazepam, clonazepam), hypnotics (zopiclone, zolpidem), sedatives (neuroleptics, some antihistamines) or anaesthetics (GHB, ketamine), drugs of abuse, such as cannabis, ecstasy or LSD or, more often, ethanol. Mistreatment of older people, whether it is abuse or neglect, can be classified as physical, psychological, or financial/material. Several types of mistreatment may occur simultaneously. Very few data are available in the international literature. It seems that mental abuse and neglect are more frequent, but physical abuses such as beating, pushing, kicking and possibly sexual abuses have also been reported.

Method: Drugs used to facilitate sexual assaults can be difficult to detect (active products at low dosages, chemical instability), can possess amnesic properties and can be rapidly cleared from the body (short half-life). In these situations, blood or even urine can be inadequate. This is the reason why some laboratories have developed an original approach based on hair testing.

Results: Hair was suggested as a valuable specimen in situations in which, as a result of a delay in reporting the crime, natural processes have eliminated the drug from typical biological specimens. Hair analysis may be a useful adjunct to conventional drug testing in sexual assault. It should not be considered as an alternative to blood and urine analyses, but as a complement. MS/MS technologies appear to be required for analyses in drug-facilitated cases. The experience of the authors will be presented in cases involving the elderly and chemical poisoning with alprazolam, diphenhydramine, doxylamine, clonazepam, flunitrazepam and promazine.

Keywords: hair, drug-facilitated crime, age, elder abuse

38. Identification of alprazolam in a suspected drug-facilitated sexual assault

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Introduction: A juvenile reported receiving small white pills prior to numerous sexual assaults over a four month period. Investigation uncovered alprazolam as a possible drug given to the juvenile. There was no history of alprazolam being prescribed to the juvenile. Hair was collected from the child and submitted to our laboratory for alprazolam testing.

Methods: Existing benzodiazepine immunoassay screens used in our laboratory for urine were evaluated for hair matrices. One kit demostrated superior results for alprazolam in hair, so it was fully validated using hair as the matrix. The limit of detection for alprazolam in hair was determined to be 40 picograms/milligram (pg/mg) of hair, with a 25 milligram sample size. Validation included analysis of five sources of blank hair, as well as hair spiked with alprazolam at known concentrations on three separate days. Confirmatory analysis of alprazolam in hair was also validated for this case. Our laboratory's liquid chromatography/tandem mass spectrometry (LC/MS/MS) procedure for benzodiazepines was altered to target alprazolam and its correlating deuterated internal standard. Hair samples were ground to a fine powder using a bead beater and incubated overnight in methanol after the addition of 2.5 nanograms of d_5-alprazolam (100 pg/mg). Methanol extracts were concentrated and analyzed by LC/MS/MS. Separation was performed on an Alltech Altima C18 analytical column (15 cm x 2.1 mm x 5 µ) using a mobile phase of methanol, water and ammonium hydroxide (60:40:0.03). Analysis by LC/MS/MS was performed by electrospray using an LTQ mass spectrometer by Thermofinnigan. A detection limit of 20 picograms alprazolam per milligram of hair was determined by analyzing hair spiked

with alprazolam in triplicate on two days. Ten sources of blank hair were also analyzed to evaluate interferences.

Results: Using a combination of immunoassay and LC/MS/MS, alprazolam was qualitatively identified in hair from a juvenile victim in a suspected drug-facilitated sexual assault. Segmental analysis of 2-cm segments identified alprazolam in the hair correlating to growth during two of the four months that the alleged victim reported being drugged.

Conclusion: With minor modification to existing methods for benzodiazepines in urine, and several days of validation using spiked hair samples, suitable methods for alprazolam in hair were developed. The combination of immunoassay techniques and tandem mass spectrometry provided unequivocal identification of alprazolam in the hair sample of an alleged victim of drug-facilitated sexual assault.

Keywords: alprazolam, drug-facilitated sexual assault, hair analysis

39. Correlation of postmortem toxicology results with hair analysis in cases of suspected acute drug intoxication

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Introduction: The use of hair as an alternative matrix for drug testing is becoming more common. The principal advantage of hair over traditional biological specimens is that detection times are longer potentially allowing the identification of a wider spectrum of compounds and drug-use history. In postmortem toxicology the analysis of hair may be especially useful in cases where blood or other fluids are not be available. A disadvantage is that it may take up to a week for drugs to appear in hair after acute use. However, hair may be useful in determining the chronicity of drug use in acute drug deaths potentially affecting interpretation. This study examined postmortem toxicology results with hair in 14 medical examiner cases where acute drug intoxication was suspected based on scene investigation and case history.

Methods: Routine toxicology testing was performed at the Wayne County Medical Examiner's Office (WCMEO) using a combination of ELISA and/or GC-NP and GC-MS. Hair samples were blinded and sent to Immunalysis for analysis. Hair samples were screened by ELISA for carisoprodal, cocaine, benzodiazepines, fentanyl, methadone, opiates, oxycodone, tramadol, propoxyphene, amphetamine and methamphetamine. Confirmatory testing in hair was limited to the drug classes screened using either GC/MS, two-dimensional GC/MS or LC/MS/MS.

Results: The incidence of positive results (for the drugs that were tested in both traditional specimens and hair) for the 14 cases are presented below:

Confirmed Drugs	Trad. Samples	Cutoff*	Hair	Cutoff (IA)*
Cocaine, BZE, CE	3 / 4 / 0	100 (IA)	12 / 12 / 4	500
Morphine / 6-AM	8 / 5	50 (IA)	10 / 11	200
Codeine / Hydrocodone	7 / 4	50 (IA)	10 / 8	200
Oxycodone	2	25 (MS)	6	300
Benzodiazepines	5	100 (IA)	6	200
Methadone	1	12 (MS)	3	200
Fentanyl	4	2 (IA)	4	20
Tramadol	1	12 (MS)	1	1000
Propoxyphene	0	12 (MS)	1	200
Carisoprodal	0	1000 (MS)	1	1000

* Cutoff (biofluids (ng/mL), hair (pg/mg)) for initial test; IA=immunoassay, MS=GC/MS

Conclusions: With the exception of fentanyl in one case, all drugs detected in traditional specimens were also detected in hair, sometimes at very high concentrations suggesting, at least for these cases, that the majority of acute drug deaths are associated with chronic prior use of that drug. In theory, this could be useful information, especially for prescribed drugs subject to tolerance such as methadone. However, in the cases presented here, hair testing did not result in a change in the cause or manner of death. By virtue of longer detection times, the frequency of detection of drugs screened in hair was considerably greater than in traditional samples. This may provide a greater context to case history and impact the determination of cause and manner of death in natural and undetermined deaths.

Keywords: hair, postmortem, acute intoxication

40. Kinetics of disappearance of cocaine from hair after discontinuation of drug use

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Introduction: Methods that employ detection of various drugs in hair, in particular cocaine, have become popular in forensic medicine in recent years. An important use of these methods is monitoring of compliance with drug abstinence in clients of Children Protection agencies (usually parents of children). Nevertheless, the mechanisms and timeframe of drug disappearance from hair are not well characterized, but its understanding is crucial for clinical and forensic application of hair testing. Our aim was to evaluate the kinetics of disappearance of cocaine from hair after discontinuation of drug use.

Methods: The Motherisk laboratory at the Hospital for Sick Children in Toronto routinely receives hair samples for toxicology analysis from various hospitals and children's aid societies (CAS) throughout Canada. Results of cocaine and benzoylecgonine (BE) hair analyses were obtained from the Motherisk Database for calculation of half-life of these compounds in hair. Patients were included in the study if they had gradually decreasing concentrations of cocaine and/or BE in sequential hair samples, with higher levels in the distal segments (i.e. earlier in time) and low or non-measurable levels in the segment, closest to the scalp (i.e. closer to the date of sampling). No information regarding actual patterns of drug use was stored in the database, but it is conceivable that patients referred by CAS would be under large pressure to discontinue drug use. The study was anonymous, and received ethics approval by the Ethics Review Board of the Hospital for Sick Children. Half life of cocaine and BE in hair was calculated using standard pharmacokinetics calculations. Regressions and comparisons were conducted by Mann Whitney U test and Spearman rank analysis, as appropriate.

Results: Results of 137 patients fulfilled the inclusion criteria for the study. The median half life of cocaine in hair was 1 – 1.2 months in females and 1.1 – 1.3 months in males. The median half life of BE was 0.9 – 1.2 in females and 1 – 1.3 in males. Half lives of cocaine and BE were not statistically different between males and females (Wilcoxon Rank test; P=0.93 for cocaine, P=0.99 for BE). Half lives of cocaine and BE were strongly correlated (Spearman Rank rho=0.73; p<0.001).

Conclusion: Cocaine and its metabolite BE could be detected in hair of former drug users for several months after abstinence was started. The calculated half life of over 1 month for cocaine implies that, depending on the initial concentration, at least 3 – 4 months have to pass for hair testing to become negative in the segment proximal to the scalp. This finding has important implications for monitoring of compliance with abstinence of former drug users, and suggests that caution has to be exerted when estimating breach of abstinence.

On the other hand, the nature of hair testing, which can only evaluate drug exposure of moderately large periods, would mean that abstinence may not be easy to differentiate from low level exposure in the first months after abstinence was started.

Keywords: hair testing, cocaine, half life

41. Assessment of passive exposure to cocaine in preschool children from a Mediterranean city by hair testing

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Introduction: At present cocaine is the principal drug of abuse consumed in the Mediterranean basin. In domestic environments of cocaine users children, particularly the preschool ones, may be chronically and repetitively exposed to cocaine by second-hand drug smoking, accidental drug ingestion, physical contact with the users or with contaminated house surfaces. Hair testing of eventually exposed children can provide information related to a retrospective wide time window. We determined the prevalence of passive exposure to cocaine and the association of exposure with objective physical findings in children presenting to the pediatric emergency department at Hospital del Mar in Barcelona, Spain without signs or symptoms suggestive of the exposure.

Methods: Hair samples were obtained by 181 children between more than 12 months and 5 years of age. Younger children were excluded since the eventual presence of cocaine in their hair could be partly due to in utero exposure to drug. Hair samples were analyzed for the detection of cocaine and benzoylecgonine (BZE), together with opiates and amphetamines by a standardized procedure based on gas chromatography-mass spectrometry. Parental sociodemographics and drug history and child birth weight were recorded. All the children were clinically examined.

Results: Preliminary results on 32 hair samples showed 8 cases positive to cocaine (concentration range: 0.5 - 5.96 ng/mg hair) and/or BZE (concentration range:0.5- 2.45 ng/mg hair), one of which was also positive for MDMA (0.5 ng/mg) and MDA (0.3ng/mg). The parents of 5 out of the 8 exposed children tested positive for hair cocaine. In case of the other 3 children, parents were not present at the emergency room and hair collection was not possible. There was no association between parental sociodemographics and exposure to cocaine. None of the children presented with a complaint or was identified as having clinical problems currently associated with exposure to cocaine.

Conclusion: Among the inner-city children served by Hospital del Mar, it seems that significant numbers of infants and young children are being exposed to cocaine. Hair testing for cocaine and other drugs of abuse should be considered in case of children from suspected drug-abusing parents for medical follow-up, social intervention and eventual discontinuation of children from risky environments.

Keywords: cocaine, hair testing, preschool children

TIAFT Meeting

42. History of forensic toxicology: from the early days to TIAFT

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Poisons were used for different purposes since more than 5000 years. Many mineral poisonsas well as toxins from plants or animals have been utilized by mankind to kill individuals orto organize mass poisoning. Since Paracelsus (1493-1541) understanding of toxicology hasbeen progressively improved.

From the ancient Greek civilization, via the Italian school of poisoners and the Paris school of poisoners, to the 19th century, where criminal poisoning was by far more common than nowadays, a progressive improvement of investigation tools by forensic toxicology has been observed.

As an illustration of the development of modern toxicology since Orfila, some famous criminal poisoning cases from all over the world will be summarized with emphasis on the victims, the motivation, the geographical location, the poisons used, the testifying toxicologists (if known) and the outcome of the criminal poisoning cases: Barlow, Becker, Besnard, Bocarmé, Buchanan, Crippen, Girard, Lafarge, Lehmann, Marec, Nozières, Shipman cases.

Over the years toxicologists requested to give evidence in critical cases were challenged by defence lawyers during court trials. As a consequence substantial improvements of forensic toxicology have been initiated.

Since 1963 the foundation year of TIAFT, a considerable amount of efforts by mutual stimulation have been put in the development of analytical toxicology. Alternative matrices for biological sampling have been introduced. New analytical technologies have been developed allowing to use reliable methods to identify and quantify many toxicologically relevant substances with very low limits of detection and quantification. In TIAFT quality assurance aspects, collection and storage of evidence aspects, pathology of poisoning aspects, ethical and social aspects among its members have also taken an important part of its activities.

Due to better knowledge of toxicants' disposition, metabolic transformations and action mechanisms, better understanding of genetically determined polymorphisms among individuals, interpretation of toxicological findings has been significantly improved over the
last 40 years to be useful *in foro*.

Keywords: history, forensic toxicology, TIAFT

43. Is rum toxic?

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Introduction: During his second trip to the Americas at the end of the 15th century, Christopher Colombus went in the West Indies with sugar cane *Saccharum officinarum*, originally coming from Asia. That is only during the 17th century, somewhere around 1640, that an alcoholic beverage, coming from the sugar cane or more exactly from molasses, a residue of the sugar cane manufacture, seems to appear in different islands of the Caribbean. A text of the middle of the 17th century deals about this brandy under the name of "wobbler" and "rumbullion" - "keep silent devil" describing the force released by its consumption. At the end of that century, designation "wobbler" seemed to disappear and the word rum - abbreviation of "rumbullion" – became commonly used.

Rum (in french rhum) is obtained by distilling the fermented cane juice (agricultural rum) or the molasses (industrial rum). Today, in Martinique every year more than 200,000 tons of sugar cane produce 8 M liters of rum and 4,500 T of sugar.

Methods: The authors describe the preparation procedure of rum in Martinique (fermentation and distilling processes), and present the various qualities of agricultural and industrial rums and explain the criteria necessary for their specific quality A.O .C label. Rum contains 40 to 50% of ethyl alcohol and is drunk pure or as a cocktail with sugar cane syrup or fruit juices. Abuse may be dangerous for health, including 2 clinical situations: those where sugar is concerned and those where alcohol is involved. Some epidemiologic studies have been conducted to sum up the situation of Martinique towards these two problems.

Results: In Martinique, diabetes of type 2 appearing in adult is the most frequent form. The prevalence of diabetes is 8 to 10%, which is 2 to 3 times higher than in the metropolitan France. Martinique is particularly exposed to excessive alcohol consumption: 14% of male population and 2.7% of female population have an addiction to alcohol. Subjects under 18 are twice as less numerous as the metropolitans to declare consuming alcohol on regular basis and four times as less numerous to declare repetitive drunkenness.

It is considered that 80 to 95% of deaths due to liver cirrhosis, alcoholic psychosis, alcoholism and upper digestive tract cancer are related to an excessive alcohol consumption. When comparing with the metropolitan France, Martinique shows a lower death rate for the liver cirrhosis and upper digestive tract cancer but a higher death rate for the alcoholic psychosis and alcoholism. This could be due to a genetic predisposition of the population.

Conclusion: Rum is a strong alcoholic beverage. Its consumption is not controlled but needs caution and people may be informed of its adverse health consequences. Therefore at the question: is rum toxic, we will answer: it depends on the use you will manage.

Keywords: rum, Martinique, manufacture, risks for health, ethanol

44. Acute intoxication with colchicine by confounding, a never ending story?

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Introduction: Colchicine is a lipophilic, potent alkaloid that can be found in the autumn crocus (*Colchicum autumnale*). It is used for the treatment of gouty arthritis as well as acute arthritis. Due to the similarity between the leaves of the autumn crocus to those of bear's garlic (*Allium ursinum*), which is a popular seasoning herb in Germany, they are frequently mixed-up. Mistakes are common especially in spring, when only the leaves can be used for the plant identification. The result of such confusion is often an acute intoxication which can lead to death. In the present case, a 65 year old Chinese woman visiting Germany was presumed to have ingested leaves of autumn crocus. She showed symptoms like sickness and stomach aches, and finally died in hospital due to a multiorgan failure.

Aim: Based on this case of acute colchicine intoxication, a new and easy method employing liquid chromatography for the identification and quantification of colchicine in plasma and several organs is presented.

Material and methods: For the quantification of colchicine a novel, simple HPLC-method was developed. Colchicine was extracted from blood and organs employing liquid-liquid extraction. Therefore 2 mL phosphate buffer (pH 6) were added to 1 mL blood. For extraction a mixture of 3 mL dichloromethane and iso-propanol (95:5 v/v) was used. After evaporation the residue was solved in acetonitrile and analysed with HPLC (mobile phase: phosphate buffer / acetonitrile (pH 2.3); column: C-18, 300 mm, Bondapak, Waters). The detection limit und limit of quantification were determined to be 4.1 ng/mL resp. 6.1 ng/mL in blood.

Results: By chemical analysis of blood samples taken in the hospital approximately 24 h after the presumed crocus ingestion, colchicine was detected in concentrations of 50 ng/mL, with the upper limit of the therapeutic range being 2.5 ng/mL. In literature, intoxications were observed at concentrations higher than 24 ng/mL. During the course of the toxicological analysis, the patient's state of health deteriorated continuously. The patient finally died, and the post-mortem concentration of colchicine was determined in femoral blood again still being at a toxic level (65 ng/mL). All tested organs contained equally high colchicine levels. Further pharmaceuticals, i.e. lidocaine, propofol, atropine and morphine were detected in femoral blood and could be traced back to having been administered in hospital. Histological examination of the liver showed diffuse vacuolization in the cytoplasma of hepatocytes. Possibly due to the relatively short survival time, no increased number of mitotic figures was found, as reported in the literature.

Discussion: Despite constant warning of poison centers and comparable institutions in Germany, the leaves of the autumn crocus still get mixed-up with those of bear's garlic. To ensure a rapid qualification and quantification in cases of suspected colchicine intoxication, we developed a reliable as well as fast and easy HPLC-method whose applicability for forensic toxicology was successfully proven.

Keywords: colchicine, HPLC, intoxication

45. Codeine in postmortem blood: c[illegible]nges for interpretation

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Introduction: Codeine is prescribed for its a[illegible] and antitussive properties, often in combination preparations with [illegible]gesics. Compared to morphine, codeine has a relatively low affinity [illegible]μ-receptors, and its analgesic (and, presumably, toxic) effects are la[illegible]endent on metabolic conversion to morphine by the polymorphic [illegible]me P450 isoenzyme 2D6. On average, approximately 10% of [illegible]e dose is metabolised to morphine, with a large interindividual [illegible]y. In light of this, it is surprising that reference literature and m[illegible]tific articles on codeine-related toxicity solely report codeine l[illegible]e have reviewed reported codeine-related deaths in four Norwegi[illegible]es (total population approx. 850,000) over a five-year period.

Methods: A total of 779 consecuti[illegible]sic postmortem toxicological cases were analysed in our laborator[illegible]n 2002 and 2007. Among these, 70 cases with detectable amounts [illegible]ne in blood were identified, of which 18 had blood concentratio[illegible] the lower threshold for toxicity according to TIAFT criteria (0.3[illegible]. Complete autopsy records for these 18 cases were obtained and rev[illegible]

Results: In the 18 cases sin[illegible] for review, femoral blood codeine concentrations ranged from 0.[illegible] mg/L. Morphine concentrations ranged from not detectable (n/d) to 3[illegible] whereas the active metabolite morphine-6-glucuronide (M6G) and [illegible]ctive metabolite morphine-3-glucuronide (M3G) were measured in [illegible]ations ranging from n/d to 0.55 mg/L and 1.5 mg/L, respectively. T[illegible]ine to codeine concentrations ratio averaged 0.07 and ranged from [illegible]ne n/d) to 0.39. Various other drugs and/or ethanol were detected[illegible]ses. Paracetamol was found in 17 of 18 cases, indicating ingestion [illegible]amol-codeine combinations. From the 18 cases, three deaths (17%) [illegible]buted to codeine intoxication, eight deaths (44%) to intoxication wi[illegible]e substances including codeine, and seven deaths (39%) to other [illegible]among eight cases with potentially lethal codeine concentrations ([illegible]L), two deaths were attributed to codeine intoxication, five to intoxic[illegible]n multiple substances including codeine, and one to other causes. [illegible]eaths ascribed to mixed intoxication where codeine was among the c[illegible]orphine was not detected (two cases) or found in a low concentrati[illegible]y not associated with toxic effects (one case).

Conclusi[illegible] findings suggest a large variability in the amount of morphin[illegible] after codeine intake in forensic cases. This would seem to repr[illegible] interpretational challenge not properly addressed in the curren[illegible]re (Gerostamoulos et al, Am J Forensic Med Pathol. 1996). Whe[illegible]nificant amount of data suggest that bioconversion of codeine to mo[illegible]essential for codeine-induced analgesia, this is not reflected in the re[illegible] overdose cases. Reports of codeine-related deaths should include c[illegible]ons of morphine and the morphine metabolites M3G and M6G.

Ke[illegible]s: codeine, forensic, toxicology

46. Fatal and severe codeine intoxication in three year old twins

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Introduction: Codeine is frequently recommended as cough and pain medication due to its low incidence of opioid-related side effects, especially in situations where airway management and neurological assessment are

critical. In spite of the frequent application to children, some authors consider that there is insufficient evidence to support the safety or efficacy of codeine as antitussive agent in this patient group. This work presents one fatal case and one case with severe intoxication of codeine in two 3 1/4 years old twins. The two boys had been administered 10 drops of a retarded codeine formulation once daily for six days, one developed severe side effects (unconsciousness, vomiting, aspiration, respiratory depression) during the night after the sixth day, the other one died in bed, while his brother was treated in the intensive care unit of our hospital.

Methods: An exhaustive analysis of body liquids and organs (serum, urine, cerebrospinal fluid and brain tissue) for codeine, morphine and their metabolites was performed by GC-MS and LC-MS/MS. A study of possible overdose due to the application of 10 drops instead of 0.5 ml using a measuring spoon was performed using pharmacokinetic predictions. Genetic polymorphism in the *CYP2D6* gen was characterised by real time-PCR using Taq Man probes. PCR coupled with fluorescence detection was used to asses the presence of CYP 2D6*3 (A 2637 Del), 2D6*4 (G 1934 A) and 2D6*6 (T 1795 Del) alleles.

Results: The codeine and morphine concentrations measured by GC-MS / LC-MS/MS were 179/174 and 33/26 ng/mL in serum, and 10 and 2.7 µg/mL (by LC-MS) in urine, respectively (for the severely intoxicated child); 547/436 and 150/139 ng/mL in serum, and 16/18.5 and 6/6.2 µg/mL in urine (in the fatal case). The concentrations of norcodeine, codeine glucuronide, normorphine, morphine-6-glucuronide and morphine-3-glucuronide were also determined. Other matrices such as brain tissue and cerebrospinal fluid were also analyzed. The therapeutic concentrations for codeine in serum are between 30 and 250 ng/mL and for morphine, between 5 and 64 ng/mL. Genotypes were identical in both twins and the results indicated that the phenotype corresponded to extensive metabolisers. Expected codeine plasma concentrations were calculated by pharmacokinetic modelling. After investigation of droplet size of the retard suspension it was observed that depending on the position of the dosing bottle, the administered dose could range from 12.5 to 23.4 mg of codeine instead of the 10 mg that should have been administered.

Conclusions: Probable causes of intoxication could be the use of drops instead of the measuring spoon, due to the variation of the drop´s weight of pharmaceutical products, or an accidentally incorrect dosage. Codeine therapy may be associated with unexpected high morphine concentrations in case of extensive metabolism by CYP2D6. In addition, reduced individual capacity in glucuronidation of codeine and morphine as a consequence of genetic polymorphism in UDP-enzymes may also give rise to elevated morphine levels.

Keywords: fatal codeine intoxication, pharmacogenetics, extensive metaboliser

47. Evaluation of morphine and their glucuronides (M6G and M3G) in opiate-related deaths

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Introduction: A significant element of a toxicological expert opinion is detection of metabolites. In the case of opiate abusers morphine and their metabolites concentrations help to establish the manner and cause of death.

Methods: The toxicological analysis based on the developed procedure with the use of liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) in the postmortem blood of opiate abusers (7 cases) collected during autopsy, which was performed within 24 hours after death.

Results: The presence of morphine (M), and glucuronides - morphine-6-β-D-glucuronide (M6G) and morphine-3-β-D-glucuronide (M3G) were demonstrated in the wide range of concentrations. Moreover, the concentration ratios M3G/M6G and M6G/M were calculated in the investigated cases. Analytical validation data are as follows: LOD=LOQ-1 ng/mL for morphine and 6- MAM; 5 ng/mL for M3G and M6G, LOL: 5 - 2000 ng/mL for M3G and M6G; LOL: 1 - 2000 ng/mL for morphine and 6-MAM.

Table 1. The toxicological findings in the investigated cases.

Cases	M ng/mL	M6G ng/mL	M3G ng/mL	M3G/M6G	M6G/M
1. BK	279	26	929	35	0.09
2. RŁ	451	42	993	23.6	0.09
3. KB	801	19	384	20.2	0.02
4. ŁM	1359	276	1082	3.9	0.2
5. CC	0	110	400	3.6	-
6. MK	103	29	268	9.2	0.2
7. AG	0.16	0.09	0.90	9.6	0.6

Conclusions: The relation of glucuronide concentration values - M3G/M6G and the ratio of M6G/M help to interpret results of toxicological expertise in the medico- legal aspect. It is possible to observe that concentration levels of these xenobiotics vary inversely with survival time of victim up to 24 hours. The concentration ratio of biologically active M3G to non active M6G seems to be very high in heroin fatalities and may be taken as a basis for classification of "rapid" (sudden) and "delayed" (with longer survival time) deaths.In fatalities concerning "delayed" death cases, a low level of free morphine and a high concentration ratio of M3G/M6G have been observed. In turn, the ratio of M6G to free morphine (M6G/M) was significantly lower in fatalities than in living abusers.

Keywords: opiate-related death, morphine glucuronides, LC-APCI-MS/MS

48. Determination of the N-Terminal Pro-BNP (NT-proBNP) in post-mortem specimens

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Introduction: In the living, different simple reliable biochemical assays can be used in the diagnosis of acute myocardial ischemia. Brain Natriuretic Peptide (BNP) and more recently N-Terminal Pro-BNP (NT-proBNP) measurements have been used for a couple of years as a valuable addition to standard clinical assessment for the identification and exclusion of acute congestive heart failure in emergency units. Classical biochemical assays in post-mortem blood, such as troponins, are not suitable for the diagnosis of death due to ischemic heart disease. Davies et al. [Am J Forensic Med Pathol. 2005; 26: 213 – 215] observed that elevated cardiac troponins are a marker of serious morbidity and are not specific for cardiac injury as primary cause of morbidity or mortality. In this study, we evaluated the interest of the use of BNP and NT-proBNP assays in different substrates obtained from post-mortem cases.

Methods: Serum were obtained after post-mortem femoral blood centrifugation. Serum, femoral blood, vitreous fluid and pericardial fluid were obtained from 96 bodies. NT-proBNP was measured using a chemiluminescent immunoassay kit (Elecsys 2010 analyzer, Roche Diagnostics) and the BNP was measured using an immunoenzymatic assay (Triage® BNP test on Triage® Meter plus, Biosite®). Patients were divided into four groups based on the diagnosis of heart disease (acute coronary thromboembolism or acute plaque rupture without evidence of chronic cardiac ischemia (A), acute coronary thromboembolism or acute plaque rupture associated with chronic coronary ischemia (B), chronic cardiac ischemia without acute coronary events (C) and control group without sign of cardiac ischemia (D))

obtained after forensic autopsy involving morphological, histological and toxicological examinations.

Results: Because BNP was not detected in any serum samples, no other investigation was conducted with other specimens. The results of NT-proBNP (pg/mL) are presented in Table 1.

Table 1. Levels of NT-proBNP in post-mortem specimens obtained from patients with diagnosis of acute coronary ischemia (A), chronic coronary ischemia (B), chronic coronary ischemia (C), and control group without sign of cardiac ischemia (D).

Groups Specimens	A (N=18) Mean±SD	B (N=13) Mean±SD	C (N=25) Mean±SD	D (N=40) Mean±SD
Serum	108±156	688±309	310±294	68±64
Femoral blood	28±36	536±310	238±198	33±30
Vitreous fluid	183±138	323±217	288±301	168±147
Pericardial fluid	1756±1718	18070±12596	5441±4408	1236±1492

Levels of NT-proBNP were significantly higher ($p<0.001$) in samples from groups B and C, than in either control group (D) and group A. Highest values were observed in samples from group B.

Conclusion: This study suggests that the determination of NT-proBNP in post-mortem specimens could be a suitable assay to reinforce the diagnosis of death due to heart disease.

Keywords: NT-proBNP, coronary ischemia, post-mortem specimens

49. Suggesting a cut-off for ethyl glucuronide in urine for forensic proof of ethanol consumption

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Objective: Ethyl glucuronide (EtG) is an ethanol consumption marker with a high sensitivity and specificity. SAMHSA recommended not to use EtG in forensic settings as it is not clear how unintended alcohol intake can affect urinary EtG concentrations. Costantino [J Anal Toxicol. 2006; 30: 659-662] reported EtG positive results after use of alcoholic mouthwash. From our experience with drinking experiments, we recommend guidelines for a reasonable use of EtG as an ethanol consumption marker.

Methods: Urine samples were collected from drinking experiments with social drinkers. EtG was determined by use of an API 365 liquid chromatography – tandem mass spectrometer with a validated method.

Results: Drinking amounts in the experiments ranged from 4.5 L German beer (150 g ethanol) in 12.5 hours down to a single administration of 0.5 g of ethanol. EtG was detectable for up to 80 hours after the consumption of 150 g ethanol, up to 12 hours after the consumption of 1.0 g ethanol and up to 10 hours after the consumption of 0.5 g ethanol. The peak urinary concentrations showed great inter-individual variations and ranged from <LOD (0.05 mg/L) to 0.35 mg/L after consumption of 1 g ethanol. After consumption of 0.5 g ethanol they were in the range of 0.06 – 0.15 mg/L.

Conclusions: Consumption of small amounts of ethanol is detectable by LC-MS/MS. Testing is performed in several laboratories in Germany, Sweden, Norway, Italy, Luxembourg, USA and proficiency tests are provided and performed on a regular basis. Positive EtG results after "lack of alcohol consumption" should not be regarded as false positives, but as a proof for uptake of alcohol, possibly in small amounts. If total abstinence is required, e.g. in alcohol withdrawal therapy, the person should completely avoid contact to ethanol in all of its forms. This requires a competent instruction of the patient during therapy about hidden alcohol. The cut-off should be increased for forensic requirements - e.g. to 0.5 mg/L – using a normalization to 100 mg/dL creatinine. In our opinion, this includes a safe-zone that is large enough to avoid false-positives. Exceeding the proposed cut-off would correspond with a consumption of more than 1 g of alcohol. We assume that this is an amount of alcohol that should be avoided by abstinent persons and cannot be taken up accidentally. In line with the "morphine in poppy seed"-problem, one can always try to override the cut-off by inappropriate consumption of large amounts of analyte-containing (or analyte-creating) substances [Westphal F. et al. Blutalkohol. 2006; 43: 14-27]. But this should rather be regarded as a problem in withdrawal therapy and a lack of education about ethanol traces in products of daily use. For proof of long-term abstinence, an additional hair-analysis for EtG and/or fatty acid ethyl esters (FAEE) should be performed.

Keywords: ethyl glucuronide, alcohol consumption biomarker, SAMHSA recommendation.

50. Is vitreous humour a useful adjunct to post-mortem blood in the investigation of heroin-related deaths?

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Introduction: Drug misuse in the North of Scotland has increased rapidly over the last decade. The number of heroin-related deaths alone is now over 40 per annum. Not withstanding the post-mortem phenomena of site-dependency and redistribution in the interpretation of a drug concentration, blood is the specimen of choice for ascertaining the pharmacological effect (therapeutic or toxic) of a drug in a deceased individual. Sometimes blood is unavailable at autopsy and it becomes difficult to identify what effect a drug may have had upon an individual. However, vitreous humour (VH) is often available. It is collected from an anatomically isolated area making it a biological fluid more resistant to putrefactive changes compared to other body fluids. Consequently, this pattern of stability makes VH an ideal specimen for toxicological study. In this study, we investigated the use of VH as an adjunct to the investigation of morphine (heroin)-related deaths.

Methods: Post-mortem blood and VH samples were taken from Procurator Fiscal cases (n=52) suspected of being heroin-related. Specimens were spiked with nalorphine (internal standard) and pre-treated with water prior to solid phase extraction. Following elution with dichloromethane:isopropyl alcohol (80:20), samples were evaporated to dryness and derivatised with BSTFA before being reconstituted in ethyl acetate and analysed by GC-MS.

Results: 6-Acetylmorphine was present in 71% of VH specimens compared to only 17% of the corresponding blood samples from the cases studied. Morphine concentrations were found to be significantly lower in post-mortem VH compared to post-mortem blood (Mann-Whitney Rank Sum Test: T=3305.5, p=<0.001). Good correlation was observed between vitreous humour and blood morphine concentrations (Pearson correlation coefficient=0.716; $p<0.001$). Linear regression related VH morphine concentrations to blood morphine concentrations according to the following equation ($p<0.001$): Blood morphine level (mg/L)=(VH morphine level (mg/L) x 1.146) +0.119 [r=0.716; 95% CI for the slope, 0.829 to 1.463; 95% CI for the intercept, 0.064 to 0.174 mg/L].

Conclusion: 6-Acetylmorphine was more frequently detected in post-mortem VH compared to post-mortem blood suggesting VH is a suitable specimen to definitively determine heroin usage. A positive correlation between blood and VH morphine concentrations was observed but the determined relationship was compounded by the presence of a proportional bias. Possible factors influencing this correlation include the distribution, stability and half-lives of 6-acetylmorphine and morphine in the two body fluids and whether the measured morphine is derived directly from heroin or indirectly from contaminant codeine (acetylcodeine) in illicit heroin. Unfortunately, we did not have information regarding the post-mortem interval for each case. The length of the post-mortem interval may affect the proportion of morphine available in the blood and VH at the time of sampling at autopsy. This introduces

the phenomena of post-mortem redistribution (PMR) and site dependency, both of which may possibly affect blood morphine concentrations more so than those in VH. Indeed, PMR may account in part for the greater degree of variation observed at higher post-mortem blood morphine concentrations. However, this difference may also be explained by the extent of diffusion of morphine between body compartments, since it would be expected that higher concentrations of morphine would be present in the blood given that heroin is usually administered intravenously.In summary, our initial findings suggest that in cases where blood is unavailable at autopsy, vitreous humour is not only a useful medium for ascertaining heroin usage but may provide a means of predicting a blood morphine concentration.

Keywords: vitreous humour, blood, morphine

51. Acute arsenic poisoning: clinical, toxicological and histopathological features

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Introduction: 30-year-old woman ingested and injected arsenic As (III) oxide in an attempt to commit suicide. Three hours later, she developed important digestive symptoms. She was transported to hospital and was transferred to the intensive care unit within 12 h of the massive injection of arsenic. Despite the therapeutic efforts, over the next 2 h she developed multiorgan failure. The post-mortem examination revealed the small and large intestines filled with aqueous liquid. Additionally, pulmonary edema and congestion of liver were ascertained. Histological examinations revealed in the myocardium a typical aspect of myocarditis, compatible with acute arsenic poisoning.

Methods: samples obtained during her brief hospitalisation (blood) and during the autopsy were analysed. There were fluid samples: urine, blood, bile and gastric content and organs samples: liver, kidney, lung and venous injection sites cubital and radial.

Toxicological analysis: ante- and post-mortem fluid samples and sections of internal organs were examined for arsenic after mineralization of samples by concentrated nitric acid (1 mL/100 mg). The residue was transferred in mobile phase. 200 µL was examined and detected. Analysis and speciation of arsenite As(III), arsenate As(V), monomethylarsonate MMA, and dimethylarsinate DMA was performed by HPLC (Waters 515) coupled with Inductively Coupled Plasma-Mass Spectrometer (ICPMS, Agilent 7500 Series).

Results: the arsenic concentrations in ante- and post-mortem fluid samples and internal organs are presented. For ante-mortem samples, we found 600 µg/L of As(III)and 39 µg/L of As(V). For post-mortem samples, we found As(V) and a metabolite DMA (dimethylarsinate) with a blood concentration of 629 µg/L (43+586), in bile 1678 µg/L (1635+43) for DMA + As(V), in gastric content 1319 µg/L of DMA. In liver DMA was 28380 µg/kg and 9870 µg/kg of As(V), in lung DMA 2780 µg/kg and in kidney DMA 12700 µg/kg, in radial venous 910 µg/kg and cubital venous 2290 µg/kg.

Discussion: Distribution of arsenic in the body and the valency was different in ante- and post-mortem samples. It was in agreement with the metabolism of arsenic (III) to arsenic (V). The toxicity of arsenic-containing compounds varies depending on the valency, the physical state of the compound and the rate of the absorption and elimination. Indeed, trivalent arsenic and its highly soluble forms are more toxic than pentavalent and nonsoluble forms. The case described concern massive poisoning with great amounts of arsenic. The clinical signs and symptoms of the deceased person compared to ante- and post-mortem arsenic concentrations in fluid samples and internal organs were consistent with acute arsenic poisoning. Our findings on the venous sites confirmed the injection of arsenic. Our results were compared with normal blood, urine concentrations and in organs of healthy people and were compared with other fatal cases of arsenic poisoning. The metabolism of arsenic can explain the toxicological results and the difference between ante- and post-mortem results. We underline the importance of multidisciplinary work to allow a comparison between ante- and post-mortem to confirm and to explain the cause and manner of death.

Keywords: arsenic, injection, suicide

52. Preliminary method for simultaneous quantification of psychoactive substances in oral fluid using UPLC-MS/MS

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Introduction: Within the European DRUID project, 4000 oral fluid samples will be collected from at random selected drivers in Belgium. These will be analysed for the presence of 30 psychoactive substances: 6-acetylmorphine, alprazolam, amitriptyline, amphetamine, benzoylecgonine, bromazepam, buprenorphine, clonazepam, 7-amino-clonazepam, cocaine, codeine, diazepam, (es)citalopram, flunitrazepam, 7-amino-flunitrazepam, lorazepam, MDMA, MDA, MDEA, methadone, methamphetamine, mirtazapine, morphine, nordiazepam, oxazepam, THC, tramadol, trazodone, zolpidem and zopiclone. Because of low sample volume (±1 mL) and the required cut-offs (low ng/mL range), an analytical method for simultaneous quantification is required.

Methods: Saliva is collected using the Statsure™ device. Target drugs are extracted using a liquid-liquid extraction based on the procedure published by Oiestad et al (Clin Chem. 2007;53:300-309). The detection method was developed using a Waters Ultra Performance LC™ system coupled to a Quattro Premier™ XE tandem mass spectrometer. A preliminary validation was performed.

Results: By using very low dwell times in combination with overlapping retention windows, the total run-time of the method (including re-equilibration) is only 7 minutes.

E-noval 2.0 was used to choose an appropriate calibration model for every compound (example given in Figure 1). LLOQ's (accuracy <20%) were derived from these models as well (e.g. morphine 0.66 ng/mL; buprenorphine 1.0 ng/mL; cocaine <0.5 ng/mL). No interfering signals above these LLOQ's were found in blank samples (n=17).

Within- and between-day CV and recoveries were determined for all compounds. (e.g. THC: between-day CV 17.7, 12.9 and 14.2%; within-day CV 15.7, 11.7 and 14.2% at low, medium and high concentrations, recovery 84%). Freeze-thaw experiments were conducted and revealed no significant instabilities. Matrix effect was determined for all compounds. ME was low (20% or less) for all compounds, except THC, buprenorphine and

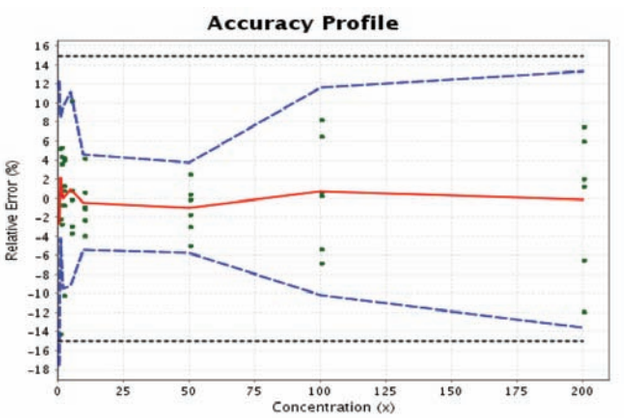

Figure 1. Accuracy profile of diazepam using weighted (1/x) quadratic regression within a range of 0,5-200 ng/mL oral fluid.

amitriptyline (80-90%), indicating ion suppression by co-eluting lipophylic compounds. Between-person CV (n=10) for matrix effect was lower than 20% for all compounds except zopiclone (45%).

Discussion: Preliminary results show that simultaneous quantification for the majority of the target compounds is possible. The method will be further optimized based on the preliminary results and will be fully validated before presentation at T2008.

53. Detection and validated quantification of the phosphodiesterase type 5 inhibitors sildenafil, vardenafil, tadalafil and two of their metabolites in human blood serum by LC-MS/MS

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Introduction: Phosphodiesterase type 5 (PDE-5) inhibitors such as sildenafil, vardenafil and tadalafil are a class of drugs used primarily in the treatment of erectile dysfunction. Sildenafil has recently also been approved for the treatment of pulmonary hypertension. The aim of this study was to develop and validate a procedure for the detection and quantification of these three drugs and some of their metabolites in human blood serum.

Methods: After liquid-liquid extraction of 0.5 mL of serum using ether-ethyl acetate (1:1) the analytes sildenafil, norsildenafil, vardenafil, norvardenafil and tadalafil were separated using a Shimadzu Prominence HPLC system with a C18 separation column (Nucleodur EC, 125/2; 3 µm from Macherey-Nagel), gradient elution with a mobile phase of 50 mM ammonium formate buffer pH 3/acetonitrile with 0.1% formic acid and a total flow of 0.5 mL/min. They were detected using an Applied Biosystems 3200 Q-Trap LC-MS-MS system (ESI, MRM mode). Calibration curves were used for quantification using sildenafil-d8 and trimipramine-d3 as internal standards. The method was fully validated according to international guidelines.

Results: The assay was found to be selective for the tested compounds. It was linear from 5 to 1000 µg/L for sildenafil, from 2 to 700 µg/L for norsildenafil, from 0.5 to 350 µg/L for vardenafil, from 0.5 to 200 µg/L for norvardenafil and from 5 to 1000 µg/L for tadalafil. The recoveries were generally larger than 50%. Ion suppression effects were not observed. Accuracy, repeatability and intermediate precision were within the required limits. They ranged from 0.1 to 20.0% for accuracy, from 1.6 to 12.2% for repeatability and from 3.8 to 17.5% for intermediate precision. The limit of quantification was 5 µg/L for sildenafil, 2 µg/L for norsildenafil, 0.5 µg/L for vardenafil and norvardenafil and 5 µg/L for tadalafil. No instability was observed after repeated freezing and thawing or in processed samples. The applicability of the assay was proven by analysis of authentic plasma samples.

Conclusion: The presented LC-MS-MS assay has proven to be applicable for determination of the studied analytes in serum. Besides routine analysis, the procedure is now being applied for quantitation of sildenafil and norsildenafil in patients taking sildenafil as medicament for treatment of pulmonary hypertension and suffering from impaired renal function or liver diseases.

Keywords: phosphodiesterase inhibitor, LC-MS, determination

54. Comparison of quantitative and qualitative drug screening approaches to forensic toxicology samples: applications for blood analyses

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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 to simultaneous screening of samples for many drugs applicable to forensic analyses. This study compared the complimentary approaches of quantitative detection with qualitative screening on an Applied Biosystems 3200 Q-Trap mass spectrometer in blood extracts. Data generation and processing was investigated with an aim to automate report generation, a common challenge for multiple analyte experiments.

Methods: Stock aqueous solutions of 107 analytes were diluted in ammonium formate (pH 3.5) and divided into 4 groups: drugs of abuse (30 drugs), antipsychotics (27 drugs), antidepressants (16 drugs), and miscellaneous drugs (34 drugs). Two MRM transitions per analyte were employed utilising the MRM catalogue database provided with the system. Analytes were combined and mixtures were separated on a Hypersil ® C18 column and gradient elution using an Agilent 1200 HPLC. The analysis was carried out in two ways. Firstly the drugs were detected using dedicated MRM mode where we looked at 2 MRM transitions per analyte. Selectivity was assessed by comparison of a number of blank blood samples and previously screened positive cases using our previously validated butyl chloride extraction process. This result was compared to a screening approach using an information dependent acquisition (IDA) experiment whereby a single MRM transition is used to trigger a qualitative full scan MSMS experiment and to match against a reference library database. **Results:** The two techniques for analysis were compared to investigate the appropriate concentration levels whereby effective screening can be carried out for a broad range of analytes. We optimised the duty cycles of the MS conditions to ensure optimal performance from a quantitative aspect, and to ensure that high quality spectral matching in the IDA experiment was maintained. Using blood extracts we designed automated reporting formats to simplify the data interrogation using Cliquid Drug Screening and Quantitation for an experiment designed to screen for a large set of forensic toxicology analytes. Matrix effects were assessed by comparison of signals of drugs without matrix and spiked in blank matrix extracts.

Conclusions: The utility of the screening experiment was demonstrated with application to blood samples. The ability to maximise the duty cycle of the experiment allowed us the option of expanding the list of analytes we could add to the screening experiment from an optimised MRM catalogue. Simplification and automation of the data generation, interrogation and reporting was found to streamline the complete analysis and assist in making the technique a routine tool in the forensic toxicology laboratory and allow for rapid turn-around of cases.

55. Combining general unknown screening by UPLC/MS and target screening by UPLC-MS/MS to improve identification of xenobiotics in biological matrix.

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Goals: The determination of xenobiotics for the investigation of chemical submission, forensic cases of suicide attempts requires methods providing both high specificity and high sensitivity like LC/MS which is now recognized as a suitable method for assessing a large panel of molecules. We have developed a fast analytical method which combines a first injection

for general screening by UPLC/MS, with a second injection for a targeted screening by UPLC-MS/MS.

Material and Method: After preparation and extraction [Humbert L. et al. IATDMCT 10th congress 9 – 14 September 2007 Nice, France] of the sample according to the matrix (blood, urine, hair), the dry extract is diluted with 100 µL of the mobile phase. The LC separation is run using an UPLC system (Waters) with a ACQUITY HSS T3 column (150 mm x 2 mm, 1.8 µm) in gradient conditions (ammonium formate 50 mM pH=3 / acetonitrile with 0.05% formic acid). The analysis time is 15 min, in multi scan single MS mode; and 15 min in MRM mode. The MS detector is a TQD (Waters), equipped with an ESI probe. The detection in single MS mode is made in multi-scan mode from 80 to 650 m/z in positive and negative ESI (4 different values of the cone voltage in ES+ and 2 in ES-). Chromatograms are automatically analyzed using the ChromaLynx software with automated matching against a 500 compound library. The second injection is an MRM method involves at least 2 characteristic transitions per molecule, with optimized parameters. Actually 322 MRMs are acquired (306 MRMs for 153 molecules, 12 MRMs for 4 molecules and 4 MRMs for 2 internal standard). The acquisitions are analyzed by QuantLynx software.

Results and discussion: In this method, the first LC/MS acquisition in multi-scan mode provides a broad screening, without prior assumption of the compounds. Sensitivity is less than in MRM, but sufficient for many molecules involved in forensic cases. The MRM detection combines a good specificity with an excellent sensitivity (for the most part less than 1 ng/mL) allowing the determination of 157 molecules some of which are toxic or lethal at low concentrations. The very short dwell times (5 ms per MRM) allows a large number of MRM's, despite narrow chromatographic peaks. This very fast (15 min for the general screening and 15 min for the targeted screening) UPLC/MS(/MS) approach permits a more complete investigation. This method allows of the determination of benzodiazepines (19 molecules), benzodiazepine-like (zolpidem, zopiclone), phenothiazines (10 molecules), diphenhydramine, colchicine… The phenomena of ions suppressions were tested, they showed increases or reductions of responses varied depending on the molecule and the matrix.

Conclusion: This concept of using 2 MS analysis methods from a single sample vial allows a broader screening, for the detection of a larger number of molecules, and an MRM acquisition for a better sensitivity. The results of the two software allow most of the time to reinforce the identification of xenobiotics through this dual detection. The UPLC/MS(/MS) combination brings speed and sensitivity, due to very sharp peaks and excellent sensitivity of the TQD at high acquisition speed. Doing both acquisitions from the same sample extract avoids repeating the preparation on samples which are sometime available in small amounts, such as hair samples.

Keywords: broader screening, target screening, UPLC-MS/MS

56. Determination of 27 opioids and their metabolites in urine by LC-MS/MS

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Introduction: The aims were to develop a validated method for the determination of 27 commonly encountered opioids and metabolites in unhydrolysed post-mortem urine. and apply it to matched autopsy urine and blood samples, to assess the value of using both matrices in interpreting the cause of death and source of the opioids detected.

Methods: Following addition of 14 deuterated internal standards, analytes were extracted by SPE with Bond Elut C18® cartridges, followed by LC-MS-MS analysis using a Thermo-Finnigan LCQ Deca Plus instrument in the ESI SRM mode, fitted with a Synergy Polar RP column (150 x 2.0 mm, 4 µm). Gradient elution used a mobile phase with (A) 10 mM ammonium formate, pH 3 and (B) acetonitrile, at a flow rate 0.3 ml/min. Analytes were identified on the basis of their retention times and the relative intensities of their pseudo-molecular ions and two product ions.

Results: Method validation: acceptance criteria for linearity, precision, and recovery were achieved for all 27 analytes. Intra-day and inter-day precision were between 1-15%. Calibration curves were linear for all analytes over the concentration range 5-250 ng/mL and correlation coefficients (R^2) were better than 0.999. LOD and LLOQ were 0.2- 0.5 ng/mL and 0.5 – 1.6 ng/mL, respectively. Recoveries were 71-111%. No interference was detected with other common drugs. Matrix effects: matrix effects on analyte ionisation were investigated using five different human urine sources at two concentrations (5 and 100 ng/mL) [Matuszewski B.K. et al. Anal Chem. 2003;75:3019-3030]. Analyte responses were within ±20% of values obtained with unextracted standards. Stability: analytes were stable under different storage conditions, apart from 6-acetylmorphine (6-MAM) and 6-acetylcodeine (6-AC) at room temperature or at 4°C. The rates of hydrolysis of 6-MAM and 6-AC in urine were low compared to blood samples. The stabilities of naloxone-3-glucuronide, dihydrocodeine-6-glucuronide, dihydromorphine-3-glucuronide and dihydmorphine-6-glucuronide in urine samples are reported for the first time. Case Samples: Matched blood and urine samples from 47 post-mortem cases and urine samples from 13 living subjects were analysed using the validated method for urine and our previously published method for autopsy blood. Total morphine to total codeine ratios were calculated to determine which opioid had been used. Also, survival time after last injection was investigated by calculating ratios between free drugs and metabolites, as described in our previous publication [Al-Asmari A. et al. J Anal Toxicol. 2007;31:394-408]. The role of opioids in combination with other drugs, including alcohol, cocaine and benzodiazepines, in opioid-related deaths is discussed.

Conclusion: A sensitive and selective method was developed for the simultaneous determination of 27 opioids and metabolites in urine. The method could differentiate between users of heroin and other opioids, such as codeine and morphine. Furthermore, urine was the sample of choice for detection of buprenorphine and norbuprenorphine and their glucuronides.

Keywords: opioids, glucuronides, post-mortem urine, LC-MS/MS, cause of death

57. Simple and rapid screening for psychotropic natural products using DART (Direct Analysis in Real Time)-TOF/MS

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Introduction: In the last decade, many analogs of narcotic substances have been widely distributed in Japan as easily available psychotropic substances. Moreover, various products of non-controlled psychotropic plants have become popular and they are causing concern.

Direct Analysis in Real Time (DART) is a novel ionization technique that provides for the rapid ionization of small molecules under ambient conditions. In this study (to investigate the trend of non-controlled psychotropic plants of abuse in Japan), a rapid screening method, without sample preparation, was developed using DART-TOF/MS for plant products composed of complicated matrixes. The major psychotropic constituents of these products were determined using LC-ESI-MS.

Methods: A variety of non-controlled psychotropic plants (gum, seeds, dried leaves, flowers, barks, *etc.*), which were advertised as "Cactus (Peyote or San Pedro)", "Salvia", "Ayahuasca", "Hawaiian baby woodrose" and "Kratom" were purchased via the Internet. For DART-TOF/MS analyses, the samples were directly placed between DART and the mass spectrometer without any extraction steps. For the LC-ESI-MS analyses, the pulverized samples were extracted with methanol under ultrasonication and filtered prior to the injection. The separation was optimized on an ODS column (Atlantis dC18, 2.1 x 150 mm, 5 µm) in an acetonitrile-10mM ammonium formate buffer

(pH 3.5) by a linear gradient program and a quantitative analysis of the major psychotropic constituents (mescaline, salvinorin A, *N, N*-dimethyltryptamine, harmine, harmaline, lysergamide and mitragynine) was carried out by the monitoring of each $[M+H]^+$ in the positive ion mode.

Results and Conclusion: As a result of the DART-TOF/MS analyses, the protonated molecular ions $[M+H]^+$ corresponding to the hallucinogenic constituents described above were detected in various plant products investigated in this study. It was possible to estimate their accurate elemental compositions through exact mass measurements. These results were consistent with those of the LC-ESI-MS analyses and the contents of the targeted compounds were in the range from 0.8 to 47 µg/mg. Typical drugs of abuse, THC, morphine and psilocin were also directly detected in marijuana cigarette, opium gum and magic mushroom respectively. Although it is difficult to estimate the matrix effects caused by other plant ingredients, the DART-TOF/MS could be useful as a simple and rapid screening method for the targeted psychotropic natural products because it provides the molecular information of the target compounds without time-consuming extraction and pre-treatment steps.

58. Synthesis and characterization of a molecularly imprinted polymer for the selective extraction of flunitrazepam and its main metabolites

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Introduction: Molecularly imprinted polymers (MIPs) are selective and synthetic sorbents possessing specific cavities, which have physical and chemical properties to recognize a target compound. During synthesis, functional and crosslinking monomers are copolymerized in the presence of a target analyte (template) in a porogenic solvent. After polymerization, the removal of the template results in well defined cavities which retain a permanent memory of the printed molecule within the polymer. The aim of this study was to synthesize and characterize a MIP for the selective extraction of flunitrazepam and its main metabolites.

Method: The polymer was thermally synthesized at 65°C during 24 hours in the presence of template (flunitrazepam), porogen (dichloromethane), an excess of monomer (methacrylic acid), a crosslinking agent monomer (ethylene glycol dimethacrylate), and radical initiator (2,2'- azobis(izobu tyronitryl)). After extraction of template, particles were packed into 1 mL cartridge for subsequent SPE utilization. Optimisation of extraction was conducted in parallel on a non imprinted polymer (NIP) to evaluate part of non selective interactions.

After enzymatic hydrolysis, each real sample has been extracted by liquid-liquid extraction with n-butylchloride and two approaches have been distinguished: a) the extract was reconstituted with mobile phase which consist of 5mM aqueous ammonium formate adjusted to pH 3 with formic acid-acetonitrile (65:35, v/v) and directly injected into the LC-APCI-MS in the positive selected ion monitoring (SIM) mode; b) the extract was reconstituted with dichloromethane and percolated onto the MIP cartridge. Afterwards, the extract was injected into the LC-MS. According to the matrix effect, the method was fully validated[1]. The developed extraction method has been then applied to real samples obtained from a voluntary man who took one pill of 1 mg Rohypnol® (flunitrazepam). Urine samples were collected during 6 days, and each of them was extracted by LLE followed by MIP clean-up.

Results: The selectivity of the MIP was demonstrated by obtained recoveries after percolation of flunitrazepam (65% on the MIP and 5% on the NIP) as well as for main metabolites including 7-aminoflunitrazepam (72% on the MIP and 33% on the NIP) and desmethylflunitrazepam (104% on the MIP and 0% on the NIP). The MIP was then applied on real urine samples. The selectivity afforded by the MIP clean-up allowed to obtained cleaner chromatograms and to eliminate all the interfering peaks present with the simple LLE. An excretion profile has been constructed allowing the determination of a unique intake of 1 mg of Rohypnol® over five days, which is much longer than without MIP clean-up.

Conclusion: A molecularly imprinted polymer has been thermally synthesised with flunitrazepam as template. Thanks to the molecular recognition, the developed polymer allowed the selectivity of the extraction to be improved and consequently the sensitivity to be enhanced. By using the polymer as clean-up tool, unique intake of 1 mg of Rohypnol® has been detected over five days. In this way, molecularly imprinted polymers would be useful in chemical submissions cases were limits of detection encountered are generally really low.

Keywords: molecularly imprinted polymer, flunitrazepam

59. What has changed in forensic analytical toxicology twenty years after the case Barschel

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Introduction: In October 11, 1987, the German politician Uwe Barschel was found dead in the bathroom of one hotel in Geneva. Six weeks later our Institute gave the results of its toxicological examinations. This primary report has been followed by several ones coming mainly from Germany.

Methods: All toxicological examinations were based on GC, GC-MS, CCM and HPLC-UV techniques.

Results: All the reports have mentioned the presence of the following compounds: cyclobarbital, pyrithyldione, diphenhydramine and perazine.

The following table summarizes the drug concentrations (mg/L) reported by our Institute in 1987 and by the Forensic Institute of Munich in 1995 (in brackets).

	cyclobarbital	pyrithyldione	diphenhydramine	perazine
gastric content	4000 (895)	200 (188)	50 (71)	75 (32)
urine	26-30 (21)	60-65 (20-61)	13-15 (36)	0.4-0.5 (0.05)
blood	40-45 (12-30)	16-18 (26-29)	0.6-0.9 (1.6)	0.2-0.3 (0.7)

Discussion: These results will be compared and discussed according to the following topics:

Methods of calibration and extraction procedures: all these results were obtained without using internal standard. In what extent the use of internal standard, as it is done now, would have improved the results?

Validation of the methods: What would have been the influence of the use of fully validated methods on the results?

Accuracy and uncertainty measurement: Are the ranges, given by the laboratories, already an estimation of the uncertainty measurement?

Stability of the compounds and conditions of sampling: From the results obtained, it seems that the stability of the four compounds and the conditions of sample were not too bad.

New samples (hair) and new techniques (HPLC-MS, HPLC-MS/MS): What would be the contribution of these new samples and techniques to a better toxicological expertise?

Interpretation of the results according to reference concentrations data found in the literature: Is it possible to give a chronology of absorption of the different drugs?

Conclusion: In the future, modern analytical instrumentation and good collections of reference postmortem data, based on much more standardized conditions, should still improve our capabilities in forensic toxicological investigations.

Keywords: Barschel case, analytical techniques, postmortem toxicology

60. Sensitive analyses of volatile organic compounds by cryogenic-oven-trapping gas chromatography-mass spectrometry

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Introduction: Volatile organic compounds (VOCs), especially toluene and xylene are widely used as solvents in industries or laboratories. These compounds are useful, however, may affect the environment and our health. VOCs are also detected in forensic cases. For example, victims of fire, suicidal or homicidal cases due to administration of chloroform, and inhalation cases of thinner were reported. Cryogenic oven trapping (COT) method has enabled the very sensitive determination of VOCs using gas chromatography (GC); we have combined COT and GC-mass spectrometry (MS) for identification and quantitation of those compounds.

Methods: Seventeen VOCs were analyzed. Deutrium-labeled diethylether or toluene was used as internal standard. Other chemicals used were of analytical grade. Human blood samples were obtained from healthy volunteers. To a 20-mL vial containing 0.5 mL of whole blood and 0.4 g of NaCl, 10 µL of VOCs mixture solution was spiked. The vial was immediately sealed with PTFE / buthyl septum cap, and heated at 60 °C for 15 min in a TuboMatrix autosampler (Perkin Elmer, USA). After that, 3 mL of headspace was injected into the GC-MS through the transfer tube. All GC-MS analyses were performed on a Shimadzu QP2010 (Shimadzu, Japan) mass spectrometer equipped with a cryogenic oven trapping system. Ionization used was EI positive ion mode. The GC column used was an Rtx-Volatiles (30 m x 0.32 mm I.D., film thickness 1.5 m, Restek, USA). The GC-MS conditions were: column temperature -30 °C (1min hold) to 200 °C (2min hold) at 20 °C /min elevation; injection temperature 240 °C; and ion source temperature 220 °C.

Results: When the column temperature was lowered at -30 °C, all VOCs including IS were sufficiently separated on the chromatogram within 12 min. Calibration curves for VOCs gave good linearity in the range of 1-2000 ng/mL except for diethylether. The detection limits (signal-to noise=3) for VOCs were estimated to be 0.2 to 1 ng/mL, and the coefficient of variation of intra-day and day-to-day analyses for VOCs at 100 ng/mL were in the range of 1.59 to 5.4% and in the range of 1.99 to 8.39%, respectively.

Conclusion: Our present method is useful for sensitive analyses of various kinds of VOCs, including lower boiling-point compound, i.e. 2-methybutane, pentane and general anaesthetics. The detection limits of the methods are similar to those of multi-SPME systems.

61. Analysis of volatile organic compounds in ambient air using HS-GC/MS after chemical desorption

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Introduction: The aim of this work was to develop a screening procedure for the detection and quantification of 43 Volatile Organic Compounds (VOC) in ambient air by HS-GC/MS after chemical desorption and its application to the measurement of workplace atmosphere.

Method: The air to be analyzed is drawn onto active charcoal cartridges by active pumping at controlled flow rate during a defined time. The VOC are trapped by adsorption on the first part of the charcoal cartridge, isolated with caps and stored at +4°C until the analyses. In a solvent free atmosphere part of the lab, the cartridges are broken and the charcoal matrix extracted by strong shaking using carbon disulfide (CS_2) in presence of the internal standard (benzene-d_6) for 1 hour. An aliquot of the solvent (0.5 mL) is diluted in water (4.5 mL) and introduced in a tube for headspace preparation (Agilent HS7694E). Tubes are warmed 20 min at 90°C, pressurized under helium (15 psi) and an aliquot is injected onto a GC/MS system (Agilent 6890N gas chromatograph coupled to a 5973 MSD). Chromatographic separation of VOC is optimized on a polyethylene glycol capillary column (30 m x 0,25 mm x 0,25 µm) using an oven temperature raising from 35 to 150°C at 5°C/min. Detection is achieved on a mass spectrometer operating in electronic impact mode of ionization at 70eV. Acquisition is realized in full scan mode for masses ranging from 30 to 180 amu.

Results: More than 40 VOC can be simultaneously detected by the developed method, from chloromethane eluted at 1.69 min to naphtalene eluated at 21.56 min, including aliphatic, cyclic and aromatic hydrocarbons (methylcyclohexane, benzene, heptane, toluene, xylenes, styrene, dichloroethane, tetrachloroethylene), alcohols (isopropanol), esters (ethylacetate, methoxyethanol), aldehydes and ketones. Quantification limits are within the range of 5 to 25 µg/tube, which is acceptable in regard to regulated exposure limit values. For example, styrene was detected at levels ranging from 4.3 to 67.1 mg/m^3 in the ambient air of an electric material industry. Several VOC (hexane, cyclohexane, acetone, tetrahydrofurane, ethylacetate, ethanol, chloroform, toluene and methylisobutylcetone) were routinely found at levels ranging from 5 to more than 100 µg/tube in the ambient air of workshop industries.

Conclusion: In our topic, ambient air testing at workplace, for occupational health and safety monitoring, can be a complementary investigation to biological monitoring. In contrast with chemical desorption which is time consuming and sometimes requires the use of toxic solvents, the more recent thermal desorption introduction technology appears as a technique that simplifies and speeds a wide range of air monitoring applications.

Keywords: VOC analysis, ambient air, solvent or thermal desorption, GC/MS

62. Analysis of volatile organic compounds by in-tube extraction (ITEX) and GC-MS

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Introduction: Screening for volatile organic compounds is an important part of comprehensive post mortem toxicology investigation. Volatile compounds can be extracted from biological material by headspace, purge and trap or headspace solid-phase microextraction. In this study, a GC-MS method was developed for volatile organic compounds in blood utilizing headspace in-tube extraction (ITEX).

Methods: Headspace extraction was performed with a CTC CombiPAL autosampler with ITEX option. Volatile compounds were enriched from 1 ml of blood by trapping into a microtrap, which was filled with Tenax and placed between the heated headspace syringe and the syringe needle. Analytes were released by thermal desorption and injected into GC-MS. All ITEX parameters were carefully validated.

GC-MS was performed with an Agilent Technologies 6890N/5975B VL MSD using a Varian CP-PoraPLOT Q capillary column (27.5 m including a 2.5 m particle trap x 0.32 mm with 10 µm film). Data processing was carried out with MSD ChemStation and using Automated Mass Spectral Deconvolution and Identification System (AMDIS). The mass detector was operated in full scan mode in the range m/z 15-550. Mass spectra of 61 volatile compounds were recorded and a spectral library was created by ChemStation. The ChemStation library was converted to AMDIS library by Lib2NIST converter. Data analysis was performed by AMDIS.

Results: The detection limits for 10 common volatile compounds in blood were as follows (µg/L): desflurane 7, diethyl ether 3, enflurane 10, ethanol 20000, ethyl acetate 10, hexane 5, methanol 3000, MTBE 20, sevoflurane 50 and toluene 30. The detection limit of acetone in water was 0.9 µg/L. Quantitative repeatability in blood was investigated by using diethyl ketone as internal standard. The precision of five parallel determinations in blood was generally good, the mean CV for different concentrations ranging from 3.8 to 7.6%. However, the linearity of the method was only satisfactory.

Conclusions: ITEX-GC-MS proved to be a simple and sensitive method for the detection of organic volatiles in blood. AMDIS software is very useful in full-scan target GC-MS screening, and target libraries are easy to build up. The software could identify compounds reliably even when no peaks could be seen in the total ion chromatogram baseline, and compounds could at least theoretically be separated when they peak within a single scan of each other.

Keywords: in-tube extraction, volatile compounds, GC-MS

63. Cocaine and metabolites in waste and surface waters across Belgium – a monitoring study

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Introduction: Cocaine abuse is currently estimated from consumer interviews, medical records and crime statistics, but these do not accurately reflect the local use. More realistic approaches based on the measurements of cocaine (COC) and major metabolites, benzoylecgonine (BE) and ecgonine methylester (EME), in waste and surface water have recently been described.

Methods: Hydrophilic interaction liquid chromatography (HILIC) and MS-MS was applied for the determination of COC, BE and EME in water samples from 41 waste water treatment plants (WWTPs) and 45 rivers across Belgium. Two 24h-composite WWTP samples (on Sundays and Wednesdays) and one river water sample were taken.

Results: COC was present in a range of <2-750 ng/L and BE in a range of <3-2250 ng/L, while EME was undetectable. Total cocaine equivalents for a region were calculated considering the flow and the number of people connected to a WWTP or covered by a river. For WWTPs, clear spatial (large drug abuse in cities *vs* countryside) and temporal (weekend *vs* mid-week) differences were observed.

Conclusion: Our data confirm the feasibility of monitoring cocaine abuse in waste and surface waters and constitute the largest surveillance campaign for COC of that type.

Keywords: cocaine, monitoring, waste water

64. The magnitude of paraoxon-induced inhibition of cholinesterase activities and pralidoxime reactivation is tissue-dependent.

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Objective: The efficiency of oximes in organophosphate poisoning is a matter of debate. We previously showed (SOT and SFTA 2007) that a single dose of pralidoxime (PRX) methylsulfate induces a complete cholinesterase reactivation while there was a complete but only transient correction of the diethylparaoxon-induced (PO) respiratory effects. This study suggests that reactivation of blood cholinesterase activity might not be associated with reactivation in target tissues. To test this hypothesis, we studied in PO poisoned rats the effect of the same PRX dose on reactivation of tissue cholinesterase activities.

Methods: Sprague-Dawley male rats (n=5) were poisoned using PO (0.215 $mg.Kg^{-1}$ corresponding to 50% LD_{50}) administered subcutaneously. Rats were treated with PRX (base: 50 $mg.Kg^{-1}$, IM) 30 min post injection of PO. Rats were sacrificed 90 min after PRX using a dioxide chamber. Whole blood specimens were sampled and immediately diluted (1/20) in distilled water. Rats were washed-out from blood using saline isotonic perfusion by cannulation of aorta. Tissues (diaphragm, lungs, brainstem, thigh muscle, brain frontal area) were immediately taken. All samples were kept to –80°C until the time of determinations. At the time of dosage, tissues (about 200 mg) were crushed in isotonic saline with Triton X100 (1% v/v), then centrifuged. Total cholinesterase activities were measured by radiometric assay in diluted whole blood and in tissue supernatants. Cholinesterase activities were expressed as IU. g^{-1} of hemoglobin for whole blood and in $UI.g^{-1}$ of proteins for tissues. Results are expressed as median of per cent of residual activities compared to control group [5 – 95 percentile] Statistical analysis was performed using ANOVA nonparametric tests with $p<0.05$.

Results: Comparing to the control group, PO poisoning induced a significant inhibition ($p<0.01$) in whole blood cholinesterase activity (residual activity: 54% [49 – 59]) and in tissues (residual activities, brainstem: 76% [69 – 83]; lungs: 66% [56 – 75]; diaphragm: 58% [52-64], and thigh muscle: 60% [52 – 68]) except for the frontal area in which 94% [90 -98] of residual activity remained. Ninety minutes after PRX administration, a complete reactivation of whole blood cholinesterase (95% [94 -96] residual activity) was observed. In tissues, PRX induced a complete reactivation of cholinesterases in brainstem (residual activity: 97% [96-98]) but only partial in other tissues, ranging from 76% [68-84] ($p<0.01$) (diaphragm) to 84% [78 – 91] ($p<0.05$) (lungs), with intermediate reactivation for thigh muscle 78% [65 – 91]) ($p<0.01$) that still remained different from control values. PRX was devoid of any effects in the brain frontal area.

Conclusion: This study showed that PO-induced inhibition of cholinesterase activities was tissue dependent, including the whole blood, and, within the same tissue (brain), area-dependent. Consequently, tissue cholinesterase activities were not well correlated to blood. This study was limited to PO and caution is required regarding extension of the results to other organophosphates. PRX induced a complete recovery of whole blood cholinesterase activity. In contrast, PRX induced a partial recovery that still remained significantly lower than controls in tissues in close contact with blood including diaphragm thigh muscle and lungs. Surprisingly, despite the blood brain barrier, PRX induced a complete reactivation of cholinesterase activity in brainstem. In a previous study, we reported the rebound of PO-induced respiratory toxicity 90 min after PRX administration with normal blood cholinesterase activity. The present study showed a nearly normalization of cholinesterase activities in the different tissues with remaining differences that hardly explain the rebound of toxicity. Our results suggest that other mechanisms that inhibition of cholinesterase may account for the rebound of PO-induced respiratory toxicity.

Keywords: paraoxon, pralidoxime, tissue cholinesterases activity

65. Quantification of new generation antidepressants using a gas chromatographic-mass spectrometric method. Applications in clinical toxicology

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Introduction: According to the WHO, depression will be the second leading contributor to the global burden of disease, calculated for all ages and both sexes by the year 2020. Antidepressant (AD) monitoring in plasma is a valid tool to optimize AD pharmacotherapy for special patient populations and to determine patient compliance.

Objective: Development and validation of a GC-MS method for the new generation ADs and their metabolites in plasma for therapeutic drug monitoring purposes. During the validation, a comparison between electron (EI) and chemical ionization modes (CI) was made.

Methods: A HP 6890 GC-5973 MSD was used in SIM for the quantification of mirtazapine, viloxazine, venlafaxine, trazodone, citalopram, mianserin, reboxetine, fluoxetine, fluvoxamine, sertraline, maprotiline, melitracen, paroxetine, mcpp, norfluoxetine, des-methylmianserin, desmethylmirtazapine, desmethylsertraline, desmethylcitalopram, and didesmethylcitalopram. Fluoxetine d_6, mianserin d_3 and paroxetine d_6 were used as internal standards. The GC was equipped with a split/splitless auto-injector at 300°C and a 30m x 0.25 mm i.d., 0.25-µm J&W-5ms column. The initial temperature was set at 90°C for 1 min, ramped at 50°C/min to 180°C for 10 min, and ramped again at 10°C/min to 300°C (5 min), with a constant helium flow of 1.3 ml/min. MSD temperatures were 300°C for the transferline, 150°C for the quadrupole and 230 or 250°C for the EI or PICI-source, respectively. In NICI, the transferline was kept at 280°C, the ion source at 150°C and the quadrupole at 106°C.

Results and discussion: Sample preparation consisted of a strong cation exchange mechanism and derivatisation with heptafluorobutyrylimidazole [Wille S.M.R. et al. J Chromatogr A. 2005;1098:19-29]. The GC separation was performed in 24.8 minutes. Most ADs, as well as their heptafluorobutyryl derivatives were stable under different storage conditions. Calibrators ranged from sub till high therapeutic concentration. Calibration by linear and quadratic regression for EI and CI, respectively, utilized deuterated internal standards and a weighing factor $1/x^2$. A strong cation exchanger resulted in reproducible recoveries (72-107%) at three different concentration ranges. Intra- and inter batch precision at LOQ (1-25 ng/mL depending on ionization mode), low, medium and high concentrations fulfilled the criterion of a relative standard deviation below 20% at LOQ and below 15% at the other concentrations for most compounds. Accuracy ranged from 75-114% [Wille S.M.R. et al. J Chromatogr A 2007;1176:236-245].

Conclusion: The developed GC-MS method for the simultaneous determination of new generation ADs and their metabolites was validated in plasma. In the near future, patient plasma samples will be analysed to prove the method's usefulness in clinical settings. The TDM results will be related to the patient's CYP 2D6 profile to observe the relationship between metabolism, plasma concentrations and effect.

Keywords: antidepressant monitoring, metabolites, gas chromatography

66. Blood kinetics of ethyl glucuronide and ethyl sulphate in chronic alcoholics

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Introduction: Measurement of ethyl glucuronide (EtG) in blood has previously been suggested as a helpful tool to determine time of alcohol ingestion in for instance cases of drunk driving. Studies of healthy volunteers have shown that after ingestion of a low dose of ethanol, EtG reaches its maximum concentration 2.5-4 hours (h) after ethanol, is eliminated with a terminal half life of 2-3 h, and returns to zero about 12-14 h after alcohol ingestion. This data is not necessarily transferable to drunk drivers, where heavy drinkers are often over-represented and the consumed doses of ethanol are much higher. The aim of this study was to investigate the kinetics of EtG and ethyl sulphate (EtS) in chronic alcoholics after termination of alcohol ingestion.

Methods: Sixteen patients from an alcohol withdrawal clinic were included directly after admission. Time of end of drinking session, estimated daily intake of ethanol (EDI) and medical history was recorded. Three to five blood samples during 20-43 hours (depending on the subject's willingness and clinical status) were collected from each patient subsequent to admission. Ethanol, EtG and EtS levels were analysed in all samples. Ethanol was determined using a headspace gas chromatographic system, while EtG and EtS quantification was carried out with a liquid chromatography-mass spectrometry (LC-MS)-method. The level of quantification (LOQ) was 0.06 mg/L for EtG and 0.02 mg/L for EtS and the method was fully validated.

Results: The first samples were collected median 2.5 h after end of drinking (range 0.5-23.5). The median EDI was 228 g (70-564). Two patients had levels of EtG and EtS below LOQ in all samples, the first collected 23.5 and 9.25 h after cessation of drinking, respectively. Of the remaining 14 patients, one subject, suffering from both renal and hepatic failure, showed concentrations of EtG and EtS substantially higher than the rest of the material. This patient's initial value of EtG was 17.9 mg/L and of EtS 5.9 mg/L. He still showed a high level of EtG (3.5 mg/L) and EtS (1.4 mg/L) 31 h after end of drinking (last sample collection), and the terminal half life was calculated to 11.9 h for EtG and 12.5 h for EtS. Among the remaining 13 patients, the initial values were median 0.4 g/L (range 0-3.65) for ethanol, 1.1 mg/L (range 0.1-5.9) for EtG and 0.6 mg/L (range 0.1-1.9) for EtS. These were also the maximum values in all subjects. Elimination occurred with a median half life of 3.25 h for EtG (range 2.6-4.3). For EtS, the median half life was 3.55 h (range 2.7-5.4). EtG and EtS levels decreased to <0.3 mg/L for EtG after median 21 h (range 3.25-40) and <0.1 mg/L for EtS after median 21.4 h (range 3.25-40).

Conclusions: Kinetics of EtG in chronic alcoholics does not completely differ from healthy volunteers, and EtS seems to have some of the same qualities. A variable initial level of EtG and EtS is seen, but the elimination appears to happen in a rate comparable to results from previous studies of social drinkers. In the present work, there was one exception to this, and we suggest the renal failure, which would delay excretion of these conjugated metabolites, as an explanation. Hepatic failure could be assumed to cause a later maximum concentration of EtG and EtS, but would less probably cause the delayed excretion. Keywords: alcohol, EtG, kinetics.

67. Desalkylflurazepam – a metabolite of midazolam

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Introduction: For urine analysed with GC-MS (after acidic hydroysis) several desalkylflurazepam derivates are already described in literature. Using our LC-MS/MS procedure for general-unkown screening in a clinical case (patient 1: 1.5 year old girl) with suspicion of intoxication by any kind of sedative drug, we found midazolam (applied during intensive care), α-hydroxy-midazolam and desalkylflurazepam. Multiple Reaction Monitoring (MRM) transitions for flurazepam and 2-hydroxyethylflurazepam were not detectable.

Aim: To characterize the origin of the "flurazepam metabolite".

Methods: Serum (with buffer pH 9) was extracted with 1-chlorobutane. Urine was enzymatically hydrolyzed prior to the same extraction. Analysis was performed with LC-MS/MS using a Synergi Polar-RP 80A column (150 mm x 2 mm I.D., 4.0 µm) with a Sciex Qtrap turboionspray mass spectrometer and MRM. The validated method includes 33 benzodiazepines or metabolites, D_5-diazepam and D_4-midazolam.

Results: Results of quantitative LC-MS/MS analyses for benzodiazepines of patient 1 are shown in table 1. The patient had been admitted to hospital twice. The first stay at hospital was because of "suspicion of rota virus" (05/09/2007) without any severe symptoms. After unconsciousness at home (09/09/2007), reanimation by an emergency doctor and admission to intensive care unit, the cause for the unconsciousness was unclear and comprehensive toxicological analyses were performed from serum and urine samples, and from urine samples obtained later on: low concentrations of desalkylflurazepam were detected besides midazolam and 1-OH-midazolam (the cause of unconsciousness could not be found by toxicological analyses).

By analyzing serum from other clinical cases with midazolam-application it could be shown that desalkylflurazepam is a metabolite of midazolam

(midazolam ranged from 360 to 1600 ng/mL, desalkylflurazepam from 3.3 to 25.2 ng/mL).

Conclusions: LC-MS/MS allows the specific and sensitive analyses of a high number of substances, especially of those with high polarity, which were not amenable to GC/MS techniques without derivatisation. Previously not known minor metabolites can be found due to higher sensitivity of LC-MS/MS, and give clues to unknown metabolic pathways of a drug. In the above mentioned case it was important to exclude the uptake of flurazepam which could be done due to the lack of 2-OH-ethylflurazepam and flurazepam, and due to further investigations of blood samples of patients, who only received midazolam during intensive care treatment. Therefore an open mind to reconsider "well-known" metabolic pathways is very important while handling analytical results.

Table 1. Benzodiazepine concentrations (patient 1) in ng/mL.

CASE 1	Serum*	Serum	Urine	Urine	Urine
	05/09/2007	10/09/2007	10/09/2007	11/09/2007	12/09/2007
midazolam	n.d.	590**	45.3	218.8	883.9
desalkylflurazepam	<LOD	pos	4.7	13.6	16.2
flurazepam	<LOD	<LOD	<LOD	<LOD	<LOD
2-OH-ethylflurazepam	n.d.	n.d.	<LOD	<LOD	<LOD

*day of admission to hospital (first stay) without severe symptoms; second stay: 09/09/07: admission to intensive care unit after unconciousness at home; **detection by HPLC/DAD; n.d.: not detectable by HPLC/DAD

Keywords: desalkylflurazepam, LC-MS/MS, midazolam

68. Studies on the metabolism and toxicological analysis of mitragynine, an ingredient of the herbal drug Kratom, in rat urine using GC-MS techniques

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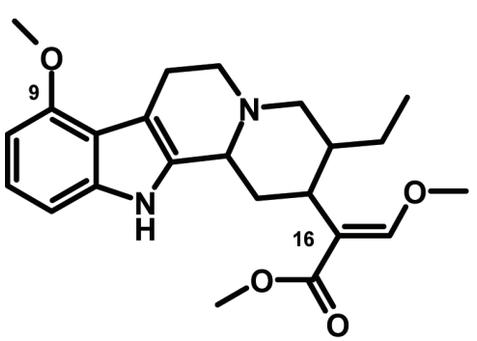
Introduction: Mitragynine (structure below) is an indole alkaloid isolated from the Thai medicinal plant *Mitragyna speciosa* (*kratom* in Thai) and reported to have opioid agonistic properties. Because of its stimulant and euphoric effects Kratom is used as herbal drug of abuse. The aim of the presented study was to identify the mitragynine metabolites in rat urine and to develop a toxicological detection procedure in urine using GC-MS.

Methods: For the metabolism study, urine samples (3 mL) from male Wistar rats, which had been administered a 40 mg/kg BW dose of mitragynine, were extracted either directly or after enzymatic cleavage of conjugates using Isolute Confirm HCX cartridges. After trimethylsilylation (TMS) the metabolites were separated and identified by GC-MS in the electron ionization mode. For toxicological detection, a 5 mg/kg BW dose of mitragynine was administered to rats and urine was collected over a 24 h period. The urine samples (3 mL) were worked-up as described above. For details see: Springer/Peters/Fritschi/Maurer, J. Chromatogr. B 789:79, 2003.



Results: Besides mitragynine, the following six metabolites could be identified in urine: 9-O-demethyl-mitragynine, 16-carboxy-mitragynine, 16-carboxy-9-O-demethyl-mitragynine, hydroxyaryl-mitragynine, hydroxyalkyl-mitragynine, and 9-O-demethyl-hydroxyalkyl-mitragynine. Based on these metabolites the following metabolic steps can be postulated: O-demethylation of the 9-methoxy group, hydrolysis of the methylester in position 16, hydroxylation of the aromatic ring or of the ethyl side chain, and combinations of some of these steps. All metabolites were partially excreted in conjugated form. Using the described detection procedure, the 9-O-demethyl, 16-carboxy, and 16-carboxy-9-O-demethyl- metabolites could be detected in rat urine within 24 h after administration of a low dose of 5 mg/kg BW of mitragynine.

Conclusion: Assuming similar metabolism, an intake of mitragynine after a common users' dose of 5-10 mg/kg BW should be detectable via its metabolites in human urine, because this human dose corresponds to a high dose of 20-40 mg/kg BW of rats according to interspecies dose-scaling.

Keywords: mitragynine, metabolism, GC-MS

69. An in vitro study on the importance of the polymorphic enzymes CYP2D6 and CYP2C19 at therapeutic and toxic levels for the metabolism of amitriptyline taking the influence of metabolites into account

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Introduction: Pharmaceuticals show large differences in the metabolic rate from person to person. For pharmaceuticals with a relatively narrow therapeutic index this can be problematic since an average dose might give a risk of intoxication in patients with a slow metabolism. Interindividual variation of the cytochrome P450 (CYP) system is the most important factor for the difference in metabolic rate. Commonly, in vitro studies on drug metabolism only deal with the parent compound and so in reality model the single-dose case only. In this present work, we studied the metabolism of the tricyclic antidepressant amitriptyline in the presence of the main metabolite nortriptyline simulating the steady-state situation with amitriptyline and nortriptyline present in the ratio 1:1. Amitriptyline and nortriptyline are equally active compounds. The metabolism of nortriptyline in the presence of amitriptyline was therefore also studied. Amitriptyline and nortriptyline are partly metabolised by the polymorphic enzymes CYP2D6 and CYP2C19. The importance of polymorphic CYP enzymes to intoxications can better be understood when precise information is available concerning the quantitative contribution of individual CYP isoenzymes. The importance of the various CYP-isoforms was here assessed at therapeutic (5 µM) and toxic (50 µM) liver concentrations. Special focus was on the role of the polymorphic CYP enzymes 2D6 and 2C19.

Methods: In vitro investigations were done with human liver microsomes (HLM) and cDNA-expressed CYP isoenzymes. Product formation was measured after an incubation period of 20 min. Analysis was done with liquid chromatography-mass spectrometry (LC-MS/MS). The importance of CYP2D6, CYP2C19 and CYP3A4 was assessed by chemical inhibition studies in HLM.

Results: At therapeutic level HLM studies show that the presence of metabolites decreases the amitriptyline hydroxylation rate by 46%, but the demethylation rate was only a little affected. At toxic level the demethylation rate of amitriptyline was decreased 46% and the hydroxylation rate decreased with 63%. The presence of amitriptyline had only a minor effect on nortriptyline metabolism rate. The importance of CYP2D6 for amitriptyline metabolism was reduced from 27% to 8% of the total metabolism rate when the concentration rose to toxic level in the steady state situation.

On the other hand, the importance of CYP3A4 grew from 19% to 42%. The importance of CYP2D6 in nortriptyline metabolism was reduced from 62% to 22%, and the importance of CYP3A4 increased from 13% to 38% when the concentration rose to a toxic level in the steady state situation. CYP2C19 showed a surprisingly low activity in the pooled HLM, but studies in a fast CYP2C19 HLM showed the same tendency of a decreased importance of CYP2C19 at a toxic concentration.

Conclusion: The results indicate that metabolites can act as competitive inhibitors at steady-state concentrations lowering the metabolism of the parent compound and changing the importance of different metabolic pathways. The importance of the polymorphic CYP isoforms CYP2D6 and CYP2C19 for the metabolism of amitriptyline and nortiprtyline diminishes as the concentration reach toxic level. This indicates that the risk of a severe amitriptyline intoxication or death because a person is a 2D6 or 2C19 poor metaboliser is unlikely, because other CYP-isoforms will be of major importance at high amitriptyline concentrations.

Keywords: amitriptyline, intoxication, polymorphic CYP

70. Isolation and purification of the 4'-hydroxymethyl metabolites of the designer drugs 4'-methyl-α-pyrrolidinopropiophenone and 4'-methyl-α-pyrrolidinohexanophenone biotechnologically synthesized using fission yeast co-expressing human cytochrome P450 reductase and human CYP2D6

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Introduction: 4'-Methyl-α-pyrrolidinopropiophenone (MPPP) and 4'-methyl-α-pyrrolidinohexanophenone (MPHP) are designer drugs with close structural relation to the scheduled stimulant pyrovalerone. MPPP and MPHP are mainly metabolized to 4'-hydroxymethyl-PPP (HO-MPPP) and 4'-hydroxymethyl-PHP (HO-MPHP), respectively, followed by oxidation to the respective carboxylic acids. For studies on the quantitative involvement of human cytochrome P450 isoenzymes in the initial 4'-methyl hydroxylation, reference standards of HO-MPPP and HO-MPHP are needed. Biotechnological synthesis had previously proven versatile for synthesis of the related metabolite 4'-hydroxymethyl-α-pyrrolidinobutyrophenone (FT Peters et al., Biochem Pharmacol, 2007). Therefore, the aim of this study was to synthesize HO-MPPP and HO-MPHP using a similar approach.

Methods: For synthesis of HO-MPPP and HO-MPHP, 250 µmol of MPPP·HCl and MPHP·HNO_3 were incubated (pH 8, 30°C) for 72 h and 66 h, respectively, with 1 L culture ($1.4 \cdot 10^8$ and $1.2 \cdot 10^8$ cells/mL, respectively) of fission yeast (*Schizosaccharomyces pombe*) strain CAD64 heterologously co-expressing human cytochrome P450 reductase and CYP2D6. After centrifugation, the supernatants were made acidic (pH 3) and subjected to solid-phase extraction (Varian Bond Elut SCX HF, 5 g, 20 mL). The eluates were evaporated to dryness and reconstituted in 13 and 8 mL of HPLC solvent, respectively. Aliquots (1000 and 250 µL, respectively) were separated by semi-preparative HPLC [Agilent G1361A 1200 preparative pump; Zorbax-300 SCX column, 9.4 × 250 mm; 50 mmol/L ammonium formate buffer (pH 3.5)/acetonitrile (80:20 v/v), 15 mL/min; UV detection at 265 nm]. After extraction of HO-MPPP and HO-MPHP from the respective basified eluent fractions with ethyl acetate and evaporation of the organic phases to approximately 2 mL, the metabolites were precipitated as hydrochlorides by adding 3 mol/L butanolic HCl. The final products were characterized by full scan GC-MS after trimethylsilylation (EI and PICI mode), ^{1}H-NMR, and HPLC-UV.

Results: In contrast to MPPP, which was only partly metabolized (about 35%), MPHP was completely metabolized, but a minor unknown metabolite (about 5%) was formed besides HO-MPHP. All four compounds were effectively extracted from the supernatants by solid-phase extraction, while the fission yeast matrix was effectively reduced. Semi-preparative HPLC using a high flow rate on the semi-preparative SCX column allowed baseline separation of HO-MPPP and MPPP as well as separation of the unknown MPHP metabolite and HO-MPHP within 15 min. The yields of HO-MPPP·HCl and HO-MPHP·HCl were 10 mg (39 µmol) and 40 mg (135 µmol), respectively. The fragmentation patterns and molecular masses observed in GC-MS were in accordance with the respective metabolite structures, which were also confirmed by ^{1}H-NMR. The product purities as estimated from HPLC-UV analysis were 93% and 99% for HO-MPPP and HO-MPHP, respectively.

Conclusion: The designer drugs HO-MPPP and HO-MPHP could be synthesized biotechnologically using human CYP2D6 heterologously expressed in fission yeast. Isolation by solid-phase extraction and purification of the raw extract by semi-preparative HPLC yielded the metabolites in high purity, so that they can be used as reference standards for future studies on enzyme kinetics.

Keywords: pyrrolidinophenones, fission yeast, metabolite

71. Microsomal synthesis of phase I metabolites of drugs and their stable isotope analogues; the identification of novel markers of ketamine administration for forensic purposes

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Introduction: *In vitro* biosynthesis using human liver microsomes was applied to help identify *in vivo* metabolites of ketamine in order to aid their detection by LC-MS/MS for forensic purposes. Further, the microsomal formation of deuterated analogues helped to verify the presence of these metabolites in urine collected from volunteers following ketamine administration.

Methods: Norketamine or norketamine-d_4 was incubated for up to 24 h with pooled human liver microsomes in phosphate buffer (pH 7.4), containing $NADP^+$, $MgCl_2$, and glucose-6-phosphate and glucose-6-phosphate dehydrogenase (for NADP+ regeneration), based on a procedure described by the microsome supplier (BD Biosciences). After protein precipitation, the supernatant was analysed by reverse phase UPLC-MS/MS. Detection was performed by MS, and MS/MS based on the precursor ions at m/z 238 (ketamine), 224 (norketamine), 222 (dehydronorketamine), 240 (^{35}Cl-hydroxynorketamine) and 242 (^{37}Cl-hydroxynorketamine). Differential extraction with chlorobutane was performed to further help identification, by adjusting the supernatant to pH 12 for extraction of basic metabolites (hydroxylated isomers of the cyclohexanone ring system, and dehydronorketamine) or pH 8.7 to also extract the phenolic metabolites (predicted isoelectric point). Deuterated analogues were added to urine collected from volunteers (n=6) following oral administration of a low-dose of ketamine (50 mg), followed by mixed-mode solid phase extraction and UPLC-MS/MS.

Results: Microsomal synthesis using norketamine as a substrate produced dehydronorketamine, and at least 7 structural isomers of hydroxynorketamine, which were chromatographically separated. Three of the isomers gave spectra indicating the presence of a phenolic group, as indicated by product spectra and this was further substantiated by the presence of these isomers following extraction at pH 8.7 but not pH 12. All metabolites detected following incubation with microsomes were also produced by the volunteers, as additionally supported by the addition of deuterated metabolites spiked into elimination samples. The periods of reliable detection for these metabolites are being evaluated to identify which are the best markers for retrospective purposes, but dehydronorketamine is a strong candidate (identified at least up to day 7).

Conclusion: The study shows the applicability of biological synthesis for the rapid preparation of metabolites of ketamine in body fluids where the compounds are commercially unavailable. To our knowledge, phenolic metabolites of ketamine have been detected for the first time. The use

of biologically-synthesized deuterated material for use as an internal chromatographic and mass spectrometric marker has not previously been investigated in the field of forensic toxicology. This approach is viable to aid the identification of metabolites, and those that are particularly of diagnostic value can be then selected as candidates for chemical synthesis of standards.
Keywords: microsomes, ketamine, metabolism

72. The role of human hepatic cytochrome P450 isozymes in the metabolism of the racemate and the enantiomers of MDMA

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Introduction: The entactogen 3,4-methylenedioxy-methamphetamine (MDMA) is mainly metabolized by demethylenation to 3,4-dihydroxy-methamphetamine (DHMA). The involvement of cytochrome P450 (P450) isozymes in this metabolic step has been studied by inhibition assays with human liver microsomes and for CYP2D6 with heterologously expressed human P450 isozymes. However, a comprehensive study on the involvement of the major P450s involved in drug metabolism has not been published yet. In addition, the chirality of this drug was not considered in these *in vitro* studies, although their *in vivo* metabolism is known to be enantioselective in humans. The aim of the present work was to study the contribution of relevant human CYP isozymes in the demethylenation of racemic MDMA and its enantiomers.

Methods: All incubations were performed with racemic MDMA and with its single enantiomers. Microsomes of baculovirus infected insect cells with ten individual cDNA expressed P450s (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5) were used in initial activity screenings for general involvement of individual P450s in MDMA demethylenation as well as experiments on enzyme kinetics of the involved P450s. Incubations were started by adding ice-cold microsomes to the incubation mixtures and terminated with aqueous perchloric acid. After adding the internal standard dihydroybenzylamine and the preservatives EDTA and $Na_2S_2O_3$, the mixtures were centrifuged and the supernatants derivatized with heptafluorobutyrylprolyl chloride in order to obtain the corresponding diastereomers. These were separated by gas chromatography and detected by negative-ion chemical ionization mass spectrometry according to Peters et al. (Clin Chem 2007) with modifications. From the acquired data, percentages of net clearance of the specific CYPs were calculated using the relative activity factor (RAF) approach (for details see Peters et al., Drug Metabol Dispos, 2008). In addition, the effect of the P450 inhibitors quinidine, omeprazole, or α-naphthoflavone on *R* and *S*-DHMA formation was assessed in incubations using pooled human liver microsomes.

Results: According to the results of the RAF approach, CYP2D6 was the most abundant P450 in metabolism and accounted for 94%, 59%, and 65% of the net clearance by demethylenation of *R,S*-MDMA, *R*-MDMA, and *S*-MDMA, respectively. Marked enantioselectivity with a preference for the *S*-enantiomers of all three substrates was observed for CYP2C19, the involvement of which in MDMA demethylenation had never been described before.

Conclusion: The isozyme CYP2C19, also enantioselective with a preference of S-MDMA *N*-demethylation (Meyer et al., TIAFT Seattle, 2007), may help to explain the lower *S*-MDMA blood levels after ingestion of racemic MDMA. In addition, this study demonstrated the importance of a complete consideration of the most important CYP isozymes in metabolism studies of xenobiotics, particularly when enantioselective metabolism is investigated.
Keywords: MDMA, cytochrome, metabolism

73. Studies on the metabolism of the Δ9-tetrahydrocannabinol precursor Δ9-tetrahydrocannabinolic acid A (Δ9-THCA-A) in rat urine using LC-MS/MS techniques

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Introduction: Δ9-Tetrahydrocannabinolic acid (Δ9-THCA-A) is the non-psychoactive precursor of Δ9-Tetrahydrocannabinol (Δ9-THC) in *Cannabis sativa*. In fresh plant material about 90% of the total Δ9-THC is available as Δ9-THCA-A and when heated or smoked, Δ9-THCA-A is partially converted to Δ9-THC by decarboxylation. As the release of Δ9-THC from its precursor Δ9-THCA-A is incomplete during smoking, Δ9-THCA-A can be detected in serum and urine of cannabis consumers. The aim of the presented study was to identify the metabolites of Δ9-THCA-A and to examine particularly whether oral intake of Δ9-THCA-A leads to in vivo formation of Δ9-THCA in a rat model.

Methods: A single Δ9-THCA-A dose (15 mg/kg body mass) was given to male Wistar rats by gastric intubation for toxicological diagnostic reasons and urine was collected separately from the faeces over a 24 hours period. The urine samples were prepared by precipitation either directly or after enzymatic cleavage of conjugates. The metabolites were separated and identified by LC-MS/MS operated in Q1-Scan-mode and subsequent Enhanced-Product-Ion-Scan analysis for gaining mass spectra. Further confirmation was achieved by accurate mass measurement using HPLC-QTOF. Additionally, the extracts obtained after solid-phase extraction and derivatization with MSTFA were analyzed by GC-MS to detect traces of potentially formed Δ9-THC.

Results: Ten metabolites of Δ9-THCA-A could be detected and partly identified by interpretation of the mass spectra obtained from the rat urine samples. According to the identified metabolites, the following main metabolic pathway can be postulated: Δ9-THCA-A undergoes a single hydroxylation to 11-hydroxy-Δ9-THCA-A (11-OH-Δ9-THCA-A), followed by conversion to 11-nor-9-carboxy-Δ9-THCA-A (Δ9-THCA-A-COOH). The parent compound and both main metabolites were also detected as the corresponding glucuronides. Additionally, one further monohydroxylated and two dihydroxylated metabolites as well as two metabolites with a keto-function were detected. Using GC-MS, besides the main Δ9-THCA-A metabolites 11-OH-Δ9-THCA-A and Δ9-THCA-A-COOH neither Δ9-THC nor its metabolites could be detected.

Conclusions: The main metabolites of Δ9-THCA-A are formed in analogy to Δ9-THC metabolism. Given the detectability of Δ9-THCA-A and metabolites in blood and urine after cannabis use, kinetic studies of these analytes could lead to new markers for recent cannabis consumption.
Keywords: Δ9-Tetrahydrocannabinolic acid A, metabolism, LC-MS/MS

74. A rapid superparamagnetic particles-based biosensor immunoassay for drugs-of-abuse in oral fluid

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Introduction: Saliva, as a biological matrix, is now widely accepted for on-site drug-of-abuse screening tests. Current products on the market are typically based on lateral flow technology and take between 5 and 15 minutes and involve numerous steps for the operator. We present here a unique and totally novel approach for the detection of drug-of-abuse in saliva. Our 5-drug

panel test can be performed in less than a minute using neat oral fluid, from sample collection to results, along with a very high sensitivity. Furthermore, the assay runs without the need of a liquid buffer to condition the sample, which significantly reduces the number of operator actions. An exceptional ease-of-use, a high sensitivity and an extremely short running time contribute to make the test particularly adequate to address the demanding requirements of road-side drug-of-abuse testing.

Methods: Our novel biosensor test is based on superparamagnetic particles (300 nm to 500 nm) with amino or carboxyl functional groups covalently linked to the monoclonal anti-drug antibodies. The assay is competitive and drug molecules from the sample compete with the drug molecules bound to the sensor surface for the antibodies binding sites. Magnetic actuation is used firstly to concentrate the magnetically labelled bio-molecules on the surface of the biosensor, hence accelerating the binding reaction, and secondly to remove unbound magnetic labels.

Results: Anti-drug antibodies were successfully linked to the superparamagnetic nanoparticles and subsequently dried without significant particle aggregation or loss of antibody activity. BSA-drugs were deposited on the sensor surface using a nanospotter and their spotting concentrations were optimised. Neat saliva was collected and guided to the sensor detection area through microfluidic channels. The baseline was established with negative oral fluid samples from voluntary donors and dose-response curves were established with negative oral fluid samples spiked with varying concentrations of morphine, benzoylecgonine, Δ^9-THC, amphetamine and methamphetamine. The average sample volume collected was 460 µL in an average time of 29 s. The overall coefficient of variation for the 5 drugs after running 30 negative oral fluid samples from volunteers was 9.3%. The stability was shown to be at least 18 months. The presence of a reference spot insured the validity of the assay.

Conclusion: Combining the principle of competitive immunoassay with our modern and unique biosensor technology allowed the performance of a sensitive saliva screening test in less than a minute for a 5 drug-panel and without any operator actions, except for inserting the collector in the cartridge and then the cartridge in the reader.

Keywords: biosensor, oral fluid, superparamagnetic particles, drugs-of-abuse

75. Prevalence of alcohol in blood samples from fatal and nonfatal traffic accident cases in Turkey

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Introduction: Alcohol is one of the main causes of traffic accidents worldwide and Turkey is one of the European countries showing highest figures for traffic accidents. Use of alcohol decreases the driving ability of an individual increasing in this way the possibilities of their involvement in traffic accidents. As a third largest city in Turkey, it is reported that Izmir is in the third place regarding the number of injured traffic accidents and in the 5th place in fatal traffic accidents. The objective of the present study was to evaluate the alcohol presence in drivers in fatal and non-fatal injured traffic accidents in Turkey.

Method: The study consisted of drivers involved in traffic, fatal (n=57) and non-fatal (n=291), accidents that occurred within the jurisdiction of Chemistry Specialization Department of the Ministry of Justice. All samples belonged to samples concerning 348 blood samples from road accident cases between February 2005 and February 2007. 83.6% (291) of the traffic accidents were non-fatal, 16.4% (57) of them were fatal.

Results: Alcohol was detected in 37% of all traffic accidents, 54.4% of fatal accidents and 34% of non-fatal injured traffic accidents. Two hundred and seventy-five (94.5%) out of the 291 samples analyzed were men and 16 (5.4%) were women. The prevalence of alcohol in woman was significantly lower that of males, in male 38.6% and in female 6.3% was positive for alcohol. As control group, 10662 (96.8%) BAC results were belonged to drivers who were were tested by breathalyzer in routine traffic controls. Alcohol was detected in 17.6% of traffic control group. In accordance with Turkish laws, subjects were considered to be positive when alcohol blood concentration exceeded 0.50 g/L. The odds ratio significantly increased with the increasing level of blood alcohol concentration (Statcalc EpiInfo version 6, $p<0.0001$).

Conclusion: In this study the value of odds ratio suggests that drivers with alcohol below legal BAC limit (BAC level with 0.21-0.50 g/L) had a 3.8 (95% C.I.=1.6–9.3) times higher risk of a fatal accident than the alcohol negative drivers. Lowering legal BAC limits in Turkey is expected to reduce the fatal traffic accidents, in particular, because accidents involving alcohol are more likely to result in fatalities.

Keywords: traffic accidents, alcohol, legal limit

76. A validated method for the extraction of drugs from the DrugWipe® drug-testing device and confirmation by LC-MS/MS

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Introduction: In recent years the demand for a fast, easy and reliable on-site drug-test has increased. Many of the drug-tests commercially available are based on immunochromato-graphical methods and are to be read visually, making the outcome very subjective [Clin Chem. 2002; 48: 174-176]. Therefore it is recommended to make a conformation analysis by GC/MS or LC-MS [Forensic Sci Int. 2001; 121: 37-46]. Additionally the available drug-tests have other drawbacks being the lack of specificity and accuracy [ROSITA-2 final report 20 may 2006]. They often give false positive or false negative results. Together with the cross-reactivity with some legal drugs this further demands the need for a confirmatory analysis of the drug-testing device when no reference material such as saliva, blood or urine is available. The DrugWipe® drug-test is a drug-test that has become popular over the past years. The test detects the most widely abused drugs on a nanogram scale and has the advantage that it can be used on saliva, sweat and surfaces. A validated method to confirm the result from the DrugWipe® drug-test will be presented.

Methods: 8 of the most commonly used drugs of abuse and some of their metabolites (cocaine, benzoylecgonine, amphetamine, methamphetamine, MDMA, MDA, morphine and codeine) were selected. The DrugWipe® drug-tests were spiked on cutoff, or near to cutoff, level with the different compounds. The drugs were dissolved in water when spiked. Additionally experiments on matrix effects were performed adding 10 µL of negative sweat or saliva prior to the extraction of the DrugWipe®. The immunochromatographic test-strip was afterwards cut into 4 pieces and extracted with 4 mL acidified methanol containing deuterated internal standards for the 8 compounds. The methanol was then evaporated under a flow of nitrogen and the residue was dissolved in 200 µL eluent and injected into the LC-MS/MS apparatus (Waters Micromass Quattro Micro) using positive electrospray ionization.

Results: All of the analyzed drugs were extracted from the DrugWipe® drug-test with recoveries at 90%. All of the analytes showed fine baseline separation that was achieved within a 12 minutes run time. They all showed good response on the LC-MS/MS apparatus. The method was linear from 5 to 500 ng/mL. Experiments on matrix effects showed no ion suppression, neither interference between the analyzed compounds. The validation showed that a positive DrugWipe® drug-test can be analyzed up to 14 days after it has been spiked regardless of the matrix.

Conclusion: The validated method is useful when a positive drug-test has to be confirmed and no reference material is available. The method extracts and identifies 8 of the most commonly abused drugs that can give a positive

result on the DrugWipe® drug-test. It also discards false positive tests when no drugs of abuse are detected. The method was successfully applied to DrugWipe® drug-tests received from local Nightclubs in Aarhus, Denmark.
Keywords: DrugWipe® drug-test, drugs of abuse, LC-MS/MS

77. Comparison of drug prevalence, sex and age distribution of the Norwegian general driving population, groups of apprehended and fatal accident drivers

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Introduction: The focus on non-alcohol drugs as a risk to traffic safety has gained increased attention during recent years. Several studies have shown prevalence of drugs among apprehended and fatal accident drivers at levels similar to alcohol. Surveys on drug use and demographic characteristics of the general driving population are necessary as control groups for risk calculations and selection of the most highly at-risk-groups for preventive action. The aim of the present study was to compare prevalence and type of drugs, age and sex distribution in three groups of drivers: 1) those stopped at random 2) apprehended drivers stopped by the police due to accident or erratic driving and 3) fatal accident drivers.

Methods: Group 1): 10816 drivers stopped at random during one year for collection of saliva samples analysed for alcohol, illegal and psychoactive medicinal drugs. Group 2): Approximately 10 000 drivers stopped by the police during one year due to suspicion of driving under the influence alcohol only (5-5500/year) or non-alcohol drugs followed by collection of blood samples (appr. 4500/year). Group 3): 243 drivers died during two years where blood samples were collected for drugs analyses. The pattern of psychoactive medicinal drug detected in the different groups of drivers was also compared to the groups of patients with prescriptions of the same drugs recorded in the Norwegian Prescription Register.

Results:

Characteristics	Gen. driving population	Apprehended drivers	Fatal accident drivers
No. (n) of subjects	10816	Appr. 10 000/year	243 /two years
Prevalence of alcohol/drugs (%)	5,7	Appr. 84	44
Alcohol only (%)	0,3	22	13
Illegal drugs (%)	1,1	> 50	19
Med. drugs (%)	4,5	> 50	20
Multi drug det. of pos. samples (%)	16	> 40%	50
Highest freq. of med. drugs (sex)	Female	Male	Male
-age (years)	> 55	20 – 29 and 30 – 29	20 – 29 and 30 – 39
- frequency (%)	8,5	25 – 30	32
Highest freq. of illegal drugs (sex)	Male	Male	Male
- age (years)	<35	20 – 29 and 30 – 39	20 – 29 and 30 – 39
- frequency (%)	2,2	25 – 30	32
Rearrest rate/earlier arrests		Appr. 50% within 3 years	Appr. 30% of drivers with drug rel. accident

Discussion: Age and sex distribution among drivers stopped at random reflected the general driving population in Norway. The pattern of medicinal drug use in different sex and age groups reflected the prescription pattern for the same compounds. Apprehended and fatal accident drivers showed similar characteristics of drug use with regards to age and sex distribution. High levels of multi drug use were frequently found in these groups compared to the general driving population. Frequent rearrests among apprehended drivers were comparable with frequent earlier arrests due to alcohol or drug related driving, among fatal accident drivers with drugs detected at the accident. Our study indicates that apprehended drivers represent a risk group different from the general driving population and patients.

78. Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of DUID

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Introduction: In recent years, the interest in the use of oral fluid as biological matrix has increased significantly. In this article, the relationship between the oral fluid and blood concentrations of drugs of abuse in drivers suspected of DUID is discussed.

Methods: Blood and oral fluid samples were collected from drivers suspected of DUID or stopped during random controls by the police in Belgium, Germany, Finland and Norway for the ROSITA-2 project. The blood samples were analysed by GC-MS or by LC-MS, sometimes preceded by immunoassay screening of blood or urine samples. The oral fluid samples were analysed by GC-MS or LC-MS(/MS).

Results: Median, mean, range and standard deviation of the oral fluid/blood ratios of the drugs of abuse analysed during Rosita-2 are given in Table 1.

Table 1. Oral fluid/blood ratios: median, mean, range and standard deviation (SD).

Substance	Median	Mean	Range	N	SD
Sum of the amphetamines	12.07	18.20	0.27 – 182.13	177	22.43
Amphetamine	13.43	19.01	0.47 – 182.13	148	22.85
MDA	4.38	5.14	1.28 – 14.61	22	3.40
MDMA	5.57	10.37	0.88 – 88.19	41	15.20
Methamphetamine	5.19	8.05	2.20 – 23.00	6	7.69
Sum of the benzodiazepines	0.04	0.59	0.002 – 19.02	48	2.77
Diazepam	0.02	0.04	0.01 – 0.15	21	0.10
Nordiazepam	0.04	0.05	0.01 – 0.23	22	0.04
Oxazepam	0.05	0.07	0.03 – 0.14	6	0.04
Temazepam	0.10	0.18	0.06 – 0.54	5	0.20
THC	15.37	34.08	0.01 – 568.91	277	63.41
Cocaine + BE	1.80	4.57	0.19 – 78.89	40	12.33
Cocaine	21.84	30.24	3.76 – 119.35	18	28.62
Benzoylecgonine (BE)	0.91	1.47	0.19 – 10.62	40	1.80
Morphine + codeine	7.17	7.16	0.91 – 13.36	14	4.34
Morphine	2.25	2.80	0.77 – 5.70	6	1.81
Codeine	9.61	10.19	0.79 – 39.0	13	9.30

Conclusion: The oral fluid/blood ratios found in our study are comparable with those that were previously published. The oral fluid/blood ratios of basic drugs such as amphetamines, cocaine and opiates are >1. The ratios for benzodiazepines were very low, as could be expected as they are highly protein bound and weakly acidic, leading to low oral fluid concentrations. The wide range of the ratios does not allow reliable calculation of the blood concentrations from oral fluid concentrations. In our study the time of last administration, the dose and the route of administration were unknown.

Nevertheless our data reflect the variability of the oral fluid/blood ratios in persons driving under the influence of drugs.

Keywords: oral fluid/blood ratio, drugs of abuse, driving under the influence

79. Intake of psychoactive drugs by DUI offenders: correlation with alcohol abuse and accident-proneness indexes

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Aim: Toxicological and forensic examinations to verify DUI offenders' capacity for driving are routinely carried out at the Section of Legal Medicine, Marche Polytechnic University, Ancona, Italy. The aim of this work was to verify if there is any correlation between intake of psychoactive drugs, as detected by anamnestic methods, and biochemical markers of liver damage, clinical signs and anamnestic-documentary data, with particular reference to accident proneness while driving. The following data were collected in the procedure:

Traditional parameters of alcohol abuse (MCV, GGT, bilirubin); identification of ethyl alcohol in urine; toxicological and forensic examination by a specialist, including family history; information on the subject's physiological, pathological, occupational and traumatic histories; an objective physical examination aimed at finding intoxication/withdrawal symptoms, and clinical data indicating alcohol abuse, together with neurological and general internal medicine tests; diagnostic procedure based on DSM-IV criteria; CDT rate, measured by capillary electrophoresis on P/ACE MDQ equipment (Beckman Coulter, USA), according to the indications and materials supplied by the manufacturer.

Methods: A population of 4315 men and women, aged between 18 and 70, whose driving licences had been suspended for DUI, were examined at the Section of Legal Medicine, in the period April 2005-November 2007. A database including 20 clinical/biochemical parameters and referred use of anxiety-reducing, anti-epileptic, hypnotic/sleep-inducing, antidepressant, antipsychotic, alcohol/drug dependence treatments was set up. Statistical analyses were carried out with the chi-square test.

Results: 284 subjects (6.58%) resulted positive for actual prescriptional drug intake. A close correlation was found between intake of psychoactive drugs and the above parameters, as follows:

slurred speech; sensory impairment; tremors; staggering gait; Romberg's sign; MCV and GGT abnormal increase; no. convictions; no. road accidents; no. workplace accidents; hepatitis	p<0.0001
abnormal pupil diameter	p<0.0006
increased sweating	p<0.0007

Conclusions. Results indicate that subjects who take psychoactive substances (benzodiazepines, anxiety-reducing, anti-epileptic and hypnotic drugs) have greater numbers of road and workplace accidents, and also a propensity to repeated DUI.

Another important correlation was found between intake of psychoactive drugs and positivity for biochemical markers of alcohol abuse (GGT, MCV).

In this category of DUI offenders the danger of the synergic action of alcohol and other psychoactive drugs is demonstrated by the higher rate of road/workplace accidents.

Keywords: psychoactive drugs; DUI offenders; accident proneness

80. Does the change of traffic law changes drunken and drugged drivers?

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Introduction: Whenever the authorities change the traffic law in order to improve traffic safety, the question arises whether this will come true or not! In Switzerland traffic regulations concerning driving under the influence of alcohol and driving under the influence of illicit drugs changed at the same time, which probably may be unique in the world. At January first, 2005 the legal limit of blood alcohol was lowered from 0.80 to 0.50 g/kg and a zero tolerance for free morphine, cocaine, THC, amphetamine, methamphetamine, MDMA, and MDEA in blood was established.

Results: In 2005, when the legal limit of blood alcohol was lowered, the number of blood alcohol samples analyzed in our laboratory dropped from 5,818 to 5,231 (-10%), but in 2006 it was again already 2.6% higher than 2 years ago. In the first months after the law changed, there was a dramatic decrease in the number of samples, e.g. minus 56% in February (fig. 1). On the other hand, the average blood alcohol concentrations remained the same (fig. 2). Due to improved rules for prosecuting drivers under the influence of illicit drugs (DUID), the number of these cases increased rapidly from 551 (2004) to 1,247 (2005; +126%), to 1,429 (2006; +159%) and to 1,689 (2007; +200%), respectively, but no statistical relevant trend was observed concerning the blood levels of the different drugs (fig. 4).

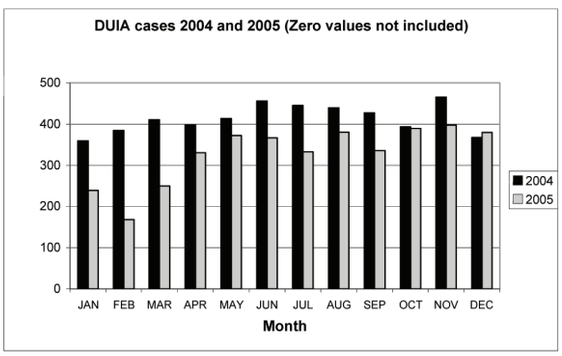

Fig. 1. Driving under the influence if alcohol (DUIA).

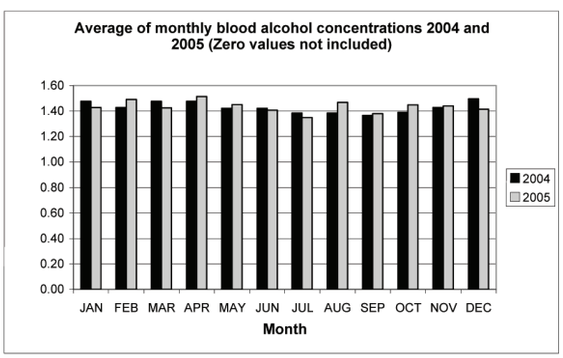

Fig. 2. No significant changes of alcohol conc.

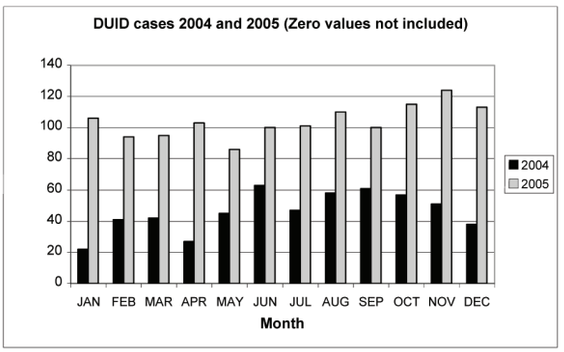

Fig. 3. Driving under the influence of drugs (DUID).

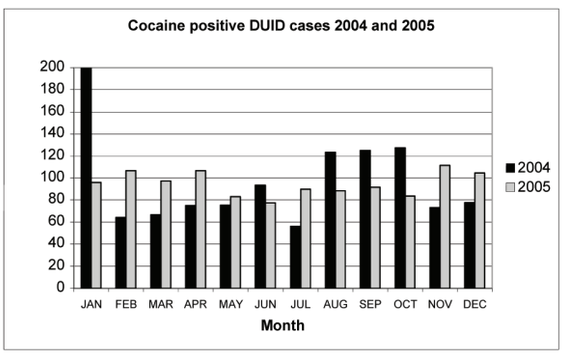

Fig. 4. No significant changes of cocaine conc. e.g.

Conclusions: After the traffic law changed, there was a significant decrease in the number of blood alcohol samples in the first year only, probably due to a transient fear of many drivers to be caught by the police. After that year things were like the years before. No changes were observed concerning alcohol and drug concentrations in blood.

81. Alcohol and drugs in fatal traffic accidents in Spain

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Introduction: It is well established that alcohol impairs driving ability and increases the risk of accident. Driving ability may also be altered by the consumption of other psychoactive substances. Because of this there is increasing interest throughout the world concerning the incidence of illicit and psychotropic drugs (pharmaceuticals) in driving and their contribution to traffic accidents. This study investigates the incidence of alcohol and drug consumption in drivers involved in fatal traffic accidents in Spain.

Methods: A total of 2761 fatally injured drivers were included in the study. 1925 (69.7%) of them were car drivers, 635 (23.0%) motorcyclists, 143 (5.2%) truck drivers, 52 (1.9%) bicycle drivers, and 6 (0.2%) bus drivers. The majority (90.1%) of the drivers were men, while only 9.9% were women. With respect to the age, the highest percentage (25.1%) was found in the group of young people from 21 to 30 years old. About 91% of the deaths occurred at the place of the accident and 9% in hospitals (in a period of less than 2 hours). About 59% of the fatalities happened on working days and 41% on weekends.

Results: The results of toxicological analyses showed that ethanol was the most prevalent substance since alcohol at or over 0.3 g/L was present at 32.3% of all drivers. Alcohol concentrations higher than or equal to 0.5 g/L were present in 27.2% of all drivers. Higher than or equal concentrations to 1.51 g/L were found in 17.5% of all deceased drivers and in 50.9% of those who tested positive for ethanol (cut-off 0.1 g/L). The highest prevalence of alcohol consumption was found in car drivers (29.4%), followed by motorcyclist (26.5%), and bicycle drivers (13.5%), and the lowest was in truckers (12.6%). Almost 7.2% of the drivers simultaneously consumed alcohol and illicit drugs. Illicit drugs (other than alcohol) were present in 4.6% of the cases, psychotropic drugs in 3.1% of the cases, and both of them in 0.7% of the cases. Cocaine was the most frequently found illicit drug (8.5%), followed by cannabis (4.2%), amphetamine and related compounds (MDA, MDMA) (0.7%), and opioids (0.4%). The main psychotropic drugs detected were benzodiazepines (3.1%), and antidepressants (1.2%).

Conclusion: The study shows how widespread the incidence of a high level of alcohol concentration was among drivers involved in fatal traffic accidents in Spain. Cocaine was the most commonly detected illicit drug.

Keywords: drugs, alcohol driving, Spain, forensic toxicology

82. A new database of chemical formulas for the screening of pharmaco/toxicologically relevant compounds in biological samples using high mass resolution

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Introduction: High mass resolution (HRMS) enables searching for a wide number of pharmaco/toxicologically relevant compounds (PTRC) in biosamples based on accurate mass and isotopic pattern measurement. However, identification power depends on the extension of reference database of chemical formulas/compound names used. Previous approaches have proposed in-house or commercial databases with limitations either in PTRC number or content (e.g. few metabolites, presence of non PTRC).

Methods: In the frame of development of a ESI-TOF PTRC screening procedure, a subset of PubChem Compound is proposed as reference database. Selection criteria for the database (ca. 50,500 compounds) are presented and its performance evaluated through analysis by capillary electrophoresis (CE)-ESI-TOF of hair/blood/urine sample collected from real positive cases.

Results: The database is rich in parent compounds of pharmaceutical and illicit drugs, pesticides, and poisons and contains many metabolites (about 6000 phase I metabolites and 180 glucuronides) and related substances (e.g. impurities, esters). The average number of compounds with identical chemical formula is 1.8±2.3 (mean±st.dev.; median=1, range 1-39). Minor deficiencies, duplicates (same compound appearing more than once in the database) and few errors (e.g. compound name associated to wrong chemical formula) do not appear to limit the potential of the database in identifying unknown PTRC.

Conclusion: The database allows a much broader search for PTRC than other previous commercial/in-house databases of chemical formulas/ compound names. However, the probability that different PTRC having identical chemical formula are retrieved is obviously higher than with smaller databases. Therefore, additional information (anamnestic/ circumstantial data, concomitant presence of parent drug and metabolite, selective sample preparation, liquid chromatographic retention and CE migration behaviour) must be used in order to focus the search.

Keywords: general unknown search, high mass resolution, chemical formula database

83. Dominance of pre-analytical over analytical variation for measurement of methadone in postmortem femoral blood

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Introduction: According to ISO 17025, it is recommended that the uncertainty of reported laboratory results is reported. Commonly, only the analytical uncertainty components are taken into account, whereas the pre-analytical component related to the sampling process is ignored. Here, we considered the total uncertainty of postmortem methadone measurements in femoral blood taking the pre-analytical component into account.

Methods: On the basis of simultaneously sampled postmortem blood specimens from the left and right femoral veins the pre-analytical variation of methadone measurements was evaluated and compared to the analytical variation. The material consisted of a series of 27 duplicate samples from routine autopsy cases comprising mainly drug addicts. A chiral LC-MS/MS method for *R*,*S*-methadone was used. The total measurement uncertainty (CV_T) was estimated from the pre-analytical variation (CV_{PA}), analytical variation proper (CV_A), and variation related to calibration (traceability) (CV_{Cal}) according to the relationship $CV_T=[CV_{PA}^{2+}CV_A^{2+}CV_{Cal}^{2}]^{0.5}$. The analytical CV was derived from QC samples. Uncertainty related to calibration concerned a component related to the purity of drug reference compound and a contribution from production of calibrator solutions. Pre-analytical sampling variation was estimated from the duplicate measurements of blood samples after subtraction of the analytical component. A 95%-uncertainty interval of a result corresponds to the interval+/-$2CV_T$.

Results: The *R*-methadone concentrations extended from 0.005 to 2.27 mg/kg with a median of 0.47 mg/kg, and the *S*-methadone concentrations ranged from 0.0005 to 1.51 mg/kg (median 0.34 mg/kg). The analytical CV was determined to be in the range 3-4% for the methadone enantiomers. Uncertainty related to calibration (CV_{Cal}) was determined to 0.60% for both enantiomers. The pre-analytical CV amounted to 18.9% for *R*- and 20.9% for *S*-methadone, i.e. considerably larger than the other components. The resulting CV_T based on squared addition of the individual components was 19.3% for *R*- and 21.2% for *S*-methadone. Thus, a 95%-uncertainty interval taking only analytical components of variation into account amounts to about +/- 7.5%, whereas a 95% - interval including the pre-analytical component corresponds to about +/- 40%, i.e. about 5 times wider.

Conclusion: Given a total standard uncertainty of 19-21%, the pre-analytical component constituted about 99% of the total CV for methadone. Dominance of the pre-analytical component of variation may be likely for other compounds measured in postmortem blood samples. Thus, the width of the 95% - uncertainty interval (+/- $2CV_T$) for a postmortem measurement is largely determined by the pre-analytical component of variation. This should be kept in mind when judging on the uncertainty of postmortem measurement results.

Keywords: pre-analytical variation, methadone, postmortem

84. On-line solid phase extraction coupled with liquid chromatography/tandem mass spectrometry simultaneous determination of opiates, cocaine and its metabolites, amphetamines and buprenorphine in serum, plasma and whole blood

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Introduction: In forensic toxicology, analytical methods should be specific and sensitive for drugs of abuse quantification in biological matrices. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is one of the methods of choice for illicit drugs quantitative determination in biological fluids. We have developed a new liquid chromatographic-tandem mass spectrometric method for determination of opiates, cocaine and its metabolites, amphetamines, buprenorphine, and glucuronide conjugates, without time-consuming sample pre-treatment.

Methods: The method uses serum, plasma or whole blood acid deproteinization and on-line solid phase extraction followed by LC-MS/MS. The system consists of two pumps for mobile phase delivery (Alliance 2795 and 1525 Micro, Waters), a six-port switching valve, an extraction column (Oasis HLB, Waters) and a reversed phase column (C18 Atlantis, Waters). The analytes are trapped on the on-line extraction column, and after the valve is switched, the retained analytes are back-flushed on the analytical column for chromatographic separation with NH_4COOH (20 mM, pH=2.8) / ACN gradient. The total analysis time for a single analysis run is 16 minutes. The mass spectrometer operates in the multiple reaction monitoring mode with atmospheric pressure positive electrospray ionization. Two transitions, optimized by infusion of individual standard solutions, were used for each molecule. Deutered analogues for all analytes of interest are used for quantification.

Results: The applied HPLC gradient ensured the elution of all the drugs examined within 16 minutes. All assays were linear within a range of 2.5 and 200 ng/mL, and limits of detection ranged from 0.1 and 1.3 ng/mL in serum, plasma or whole blood. For glucuronide conjugates, linearity ranged from 5 to 200 ng/mL and limits of detection from 2 to 3 ng/mL. The intra- and inter-day coefficients of variation were less than 15%. No ion suppression phenomenon was observed at the retention times of the drugs of abuse analyzed in serum, plasma and whole blood.

Conclusion: The present method allows on-line clean-up and enrichment, leading to improved sensitivity. This on-line solid-phase extraction LC-MS/MS method has proven to be suitable for multiple illicit drugs quantification. This method is sufficiently specific and sensitive for use in forensic toxicology compared to GC-MS, which is considered as the reference analytical method. The simple protein-precipitation step prior to on-line SPE LC-MS/MS allows reducing the sample preparation time and requires only 100 µL of biological fluids (serum, plasma or whole blood).

Keywords: LC-MS/MS, on-line SPE, illicit drugs

85. Validation of ELISA and LC-MS/MS methods for the determination of ketamine and norketamine in human urine samples

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Introduction: A recent increase in the misuse of ketamine as a recreational drug in South East Asian countries such as Taiwan, Singapore and Malaysia has necessitated the development of analytical methods for this drug. In this study, two different techniques were developed for screening and confirmation.

Method: The commercially available Neogen ELISA kit for ketamine was selected and optimised with respect to sample and enzyme conjugate volumes and the pre-incubation time. Method validation parameters investigated for ELISA were dose-response curve, intra and inter-day precision, LOD, sensitivity, specificity, and cross-reactivity studies. For confirmation, an LC-MS/MS method was developed and validated with respect to LOD, LLOQ, linearity, recovery, precisions and matrix effects. All samples were hydrolysed at 60°C for 3h using β-glucoronidase from *Helix pomatia*. Ketamine and norketamine were extracted by solid phase extraction using World Wide Monitoring Clean Screen® columns. LC-ESI-MS/MS analysis was carried out using a Thermo Finnigan LCQ Deca XP instrument and chromatographic separation was performed using a Synergi Hydro RP column. Both methods were applied to 34 human urine case samples provided by the Narcotics Department of the Royal Malaysian Police.

Results: The ELISA test was linear from 25-500 ng/mL. The cross-reactivity for the main metabolite, norketamine at 200 ng/mL ketamine was 2.1% and no cross-reactivity was detected with thirteen other common drugs at a concentration of 10,000 ng/mL. The LOD obtained was 5 ng/mL and the precision was <10%, with intraday precision (n=10) 2.47% and inter-day precision (n=10 x 5 days) 4.79% at a cut off concentration of 25 ng/mL. For the LC-MS/MS method, the LODs for ketamine and norketamine were 0.56 and 0.63 ng/mL and the LLOQs were 1.88 and 2.10 ng/mL respectively. The test demonstrated wide linearity over the range of 0-1200 ng/mL with r^2 better than 0.99 for both ketamine and norketamine. The recoveries were acceptable for both analytes at low (50 ng/mL), medium (500 ng/mL) and high (1000 ng/mL) concentrations and ranged from 97.9% to 113.3%. The method demonstrated good intra and inter-day precision (<10%). Matrix effects analysis for ketamine showed ion suppression of <10% while norketamine showed ion enhancement of <20%.

Conclusion: A simple, rapid and efficient ELISA screening test for ketamine has been optimised and validated. A complementary sensitive and precise LC-ESI-MS/MS method for confirmation has also been developed and validated. Using the cut off value of 25 ng/mL, the Neogen ELISA demonstrated a very good correlation with the LC-MS/MS method with a sensitivity and specificity of 100%. The LC-MS/MS method detected various concentrations of ketamine and norketamine in 34 human urine samples. Both methods are reliable for routine screening and confirmatory analysis of samples used in workplace drug testing and in forensic toxicology.

Keywords: ketamine, ELISA, LC-MS/MS, urine

86. Dual transition monitoring for the determination of cannabinoids in whole blood using LC-MS/MS

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Introduction: Marijuana is the one of the most commonly used illicit drugs in the world and is frequently encountered in forensic cases. Recently, recommended cut-off concentrations for the analysis of drugs in blood in driving cases were published, and the majority of forensic laboratories reported cannabis as the most frequently encountered drug. Guidelines for confirmatory analysis using LC/MS/MS suggest that the monitoring of at least two transitions is required to provide forensic identification of each drug. Since one of the main issues with the quantitation of parent THC in blood is the sensitivity required, and the monitoring of two transitions limits sensitivity, the development of an optimal analytical method has become necessary.

Methods: Whole blood specimens were screened at a cut-off concentration of 10 ng/mL using ELISA, following a 1:10 dilution and using a 25 µL sample volume. For positive specimens, cold acetonitrile (2 mL) was added to an aliquot of whole blood (1 mL), mixed and centrifuged (2500 rpm; 5 min). The supernatant was removed and internal standard was added (10 ng/mL). 0.1 M sodium phosphate buffer (pH 6.0; 1 mL) was added and the samples were extracted using StyreScreen® polymeric columns. The extracts were reconstituted in 20 mM ammonium formate pH 8.6 : methanol (50:50 v/v; 40 µL), transferred into auto sampler vials, capped and analyzed using LC-MS/MS.

An Agilent Technologies 1200 Series liquid chromatograph pump coupled to a 6410 triple quadrupole mass spectrometer, operating in positive electrospray mode was used for analysis. The LC column was a Zorbax SB C18 (2.1 x 50 mm x 1.8 µm), the column temperature was 35°C and injection volume was 5 µL. The mobile phase consisted of 20 mM ammonium formate (pH 8.6, Solvent A) and methanol (Solvent B). Initially, the mobile phase composition was 30% A: 70% B at a constant flow rate of 0.4 mL/min. After 5 min, the percentage of solvent B was 90%, remaining there for 3 min; finally at 10 min, solvent B returned to 70%. The post-time was 4 min to allow equilibrium of the mobile phase. Nitrogen gas was used as the collision gas, for nebulization (50 psi) and desolvation (300°C / 6 L/min). The capillary voltage was 3500 V. In tandem MS two transitions with one ratio calculation are considered adequate for forensic defensibility. Two transitions were selected and optimized for each drug. The transitions for THC were 315.3 to 193.1 (quantifying) and 315.3 to 122.9 (qualifying); 331.3 to 313.1 and 331.3 to 193.1 for 11-OH-THC; 345.3 to 327.1 and 345.3 to 299.1 for THCA, respectively. Each subsequent analysis required the ratio between the quantitative transition and the qualifying transition to be within +/- 20% of the ratio produced from a calibration standard (5 ng/mL), in order to be reported as positive. Since the blood samples are extracted prior to injection, using a specific procedure, matrix effects are essentially removed, making the chromatographic trace clean, and ion suppression effects minimal.

Results and conclusion: The limit of quantitation for THC was 1 ng/mL; 5 ng/mL for the two metabolites. Linearity was obtained with an average correlation coefficient for all the drugs of r^2> 0.99 over the range 0.5-100 ng/mL of whole blood. The inter-day (n=10) and intra-day (n=6) precision for the assays determined at 5 ng/mL were 9.77% and 3.87% for THC; 4.15% and 3.72% for 11-OH-THC; 9.42% and 6.27% for THCA, respectively. The method is currently in use for the analysis of authentic specimens.

Keywords: cannabinoids, whole blood, dual transition LC-MS/MS

87. A fatal cocktail on the web: helium, bromazepam and yew. A young man takes action

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Aim: We present the case of a voluntary lethal intoxication of a young man who downloaded a "method to die" on Internet. In order to prove this intoxication, we created a specific method to identify Taxine B, a major alkaloid of the European yew's leaves, Taxus Baccata, in the victim's blood sample. A 27 years old young man is discovered lying on his bed, his head resting on a pillow and his arms stretched along his body. His hands are open, palms up. A Helium bottle is found near the body, a rubber tube linked to its tap on one end and hand-taped to a torn plastic bag on the other end. When fire-fighters arrive, the bottle's tap is on. Into the young man's computer, a file is discovered: "Method to die. Bromazepam: is not lethal but the process of sleeping, unconsciousness and coma is very quick and helpful. Tejo (Taxus Baccata): lowering of heart and blood pressure, sudden death due to cardio-respiratory stop. It is a symbol of the passing to death in some cults which use it as a way to free oneself. Helium: death due to a lack of oxygen, painless. Only an external exam of the young man's body is asked by the public prosecutors' office. It is done at Paris' Forensic Institute, and a cardiac blood sample is sent to us to carry out a full toxicological analysis.

Methods: An exhaustive analysis is carried out on the blood sample. Ethanol and volatile compounds are searched and identified with HS/GC/MS, drugs are searched with a liquid/liquid extraction, LC-DAD and GC-MS. Bromazepam's specific quantitation is done once it is extracted on toxitubes A® and analysed with GC-MS in presence of nordazepam d5 as an internal standard. Several methods of extraction of the taxine B (liquid/liquid extraction with a basic pH, extraction on toxitubes A® and SPE extraction) have been evaluated in presence of an internal standard (ketamine d4). Analysis are carried out with taxine B and 2,9- diacetyltaxine B provided by the Institut de Chimie des Sciences Naturelles, CNRS.

Results: The SPE extraction gives the best results, linear regression was found in a concentration range of 0.2-500 ng/mL (for the sum of taxine B and isotaxine B), the limit of detection (LOD) was estimated at 0.16 ng/mL and the limit of quantitation at 0.4 ng/mL. The analysis of the blood sample revealed a concentration of 7.6 ng taxine /mL heart blood, calculated by the sum of taxine B and isotaxine B. Ethanol is identified in the blood sample at a concentration of 0.32 g/L and bromazepam is measured at a therapeutic concentration of 137 ng/mL.

Conclusion: In order to underline an intoxication involving ingestion of Taxus Baccata, we developed a specific sensitive method of identification taxine B in blood. This case underlines the potential hazards of Internet, in which we can find very acute information and methods to commit suicide. The fact that no autopsy was performed is regrettable. It would have allowed anatomopathological exams, notably on the lungs as they are revealing of an anoxia linked to helium respiration.

Keywords: taxine B, fatal intoxication, HPLC-MS

88. Date-rape drugs in Poland – An eight year review

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Introduction: The increase in number of reports concerning drug facilitated crimes is observed in Poland since 2000. Date-rape drugs are used for the purpose of "drugging" unsuspected victims and raping or robbing them while under the influence of the drug. In typical scenario this drug is surreptitiously added by the perpetrator to the alcoholic beverage or soft drink of an unsuspecting person. The aim of this study is to show evaluation of a scene of date-rape drugs in Poland during last eight years. Materials for this study were from the routine casework elaborated at the Institute of Forensic Research in Krakow.

Methods: APCI-LC-MS methods were applied for screening of biological materials for amphetamine and its 6 analogues (MDMA, MDA, MDE, Meth, PMA, MBDB), 13 substances from benzodiazepine group, and 42 other date-rape drugs. HPLC-DAD was used as a screening method for a wide range of medicinal drugs and NCI-GC-MS for determination of tetrahydrocannabinols (THC, 11-OH-THC, THCCOOH). Analytes were isolated from two 0.5-mL blood samples using pH dependent LLE (pH 2, diethyl ether and pH 9, ethyl acetate). The drugs were detected in combined extracts with LODs (S/N≥3) between 0.1 to 20 ng/mL. The LOQs (S/N≥10) ranged from sub-therapeutic (0.5-1.0 ng/mL, 22 compounds) or low therapeutic concentrations (2-10 ng/mL, 14 compounds) up to 500 ng/mL (42 compounds).

Results: In 2000-2007, the materials taken from 133 persons sexually assaulted and 35 perpetrators were analysed. In 2000 and 2002 not any case of this kind was registered, in 2001 only two cases were recorded. Since 2003 significant increase in the number of these cases has been observed. 11, 21, 28, 40 and 50 cases involving date-rape drugs were submitted to the Institute in 2003, 2004, 2005, 2006 and 2007, respectively. All cases were divided into two groups as victims of rapes and persons suspected for these offences. 96% of victims and 6% of suspected were females and 94% perpetrators and 4% victims were males. The most common substances detected in analysed materials collected from victims were benzodiazepines, amphetamine, tetrahydrocannabinols and MDMA. In materials collected from perpetrators the most common were tetrahydrocannabinols and amphetamine. Alcohol was found in blood and urine specimens taken from 33 persons while 68 avowed to drink alcoholic beverages. In 53% of victims and 40% of perpetrators any drug was not found. Alcohol was detected in 18% and 15%, single drug in 18% and 18%, single drug with alcohol 2% and 3%, mixture of drugs in 7% and 21%, mixture of drugs with alcohol in 2% and 3% of victims and perpetrators, respectively. A blood sample without any other evidences was the main material sent for investigation (75%). A blood sample with other materials (e.g. urine, hair, swabs, vomits, clothes, cups, tissues) were sent in 18% of cases. Other materials without the blood sample reached 7%.

Conclusion: Presented data shows that the use of drugs to facilitate sexual assaults is considerably developing in Poland. Among 153 cases involving date-rape drugs analyzed between 2000 and 2007 47% of victims and 60% of persons suspected for these offences were positive for alcohol and/or other drugs.

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Keywords: date-rape drug, drug facilitated sexual assaults

89. Identification and quantification of atractyloside (ATR) and carboxyatractyloside (CATR) by HPLC-MS2 and HPLC-MS3 in the body fluids of two rabbits poisoned by oral feeding and two actual cases of human poisoning by *Atractylis gummifera*

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Introduction: The toxic principles of *Atractylis gummifera* are diterpenic genin heterosides that inhibit the movement of the phosphorylic nucleotides ADP and ATP, preventing oxydative phosphorylation. These powerful poisons are responsible for many deaths both among humans and livestock. As well as the *Atractylis gummifera*, which is sadly only too well-known in North Africa, other sources of deadly poison are the genera *Xanthium* (Cockleburs) widespread in the Americas and China, *Callilepis laureola DC* in southern Africa.

Method: The separation was carried out on a C_{18} analytical column 150 x 2,1 mm (3,5 µm particles) using a ternary gradient composed of methanol, acetonitrile and a 2 mM formate buffer at a pH=4,5. Oxazepam D5 was used as internal standard (IS). The extraction was carried out in solid phase on an Oasis HLB® cartridge with a PBS buffer, HCl adjusted at pH=4,5.

Results: The retention times of CATR, ATR and IS are 9,6; 11,0 et 14,8 minutes respectively. In MS^3, the main ion of CATR is m/z=645, daughter of m/z=689 daughter of m/z=769 while in the case of ATR the main ion m/z=543, daughter of m/z=645, daughter of m/z=725 (negative mode). The extraction recovery is 61,6% for ATR et 59,5% for CATR.

Total quantity of ATR and CATR administered by force feeding (ng/kg)		Concentration of ATR et CATR in the body fluids according to time (ng/mL)			
		Peripheral blood		Cardiac blood	Urine
		T=6 h	T=24 h	T=24 h	T=24 h
Rabbit 1	ATR=9200	99,0	442,4	273,2	81,6
	CATR=29800	199,3	421,7	457,3	202,2
Rabbit 2	ATR=6,3	1,45	21,4	14,2	25,4
	CATR=279,6	31,1	83,0	73,9	261,1

		Concentration of ATR et CATR in the body fluids (ng/mL) of the two human poisoning cases	
		Peripheral blood	Urine
Case 1	ATR	756,1	452,4
	CATR	354,7	1855,2
Case 2	ATR	-	3,4
	CATR	-	68,9

Conclusion: This report is the first to measure the concentration of the active principles of *Atractylis gummifera L* in the body fluids of the two human poisoning cases.

Keywords: *Atraclylis gummifera*, HPLC-MS/MS, poisoning

90. Soft tissue injection of kerosene oil in a pregnant woman, a case report

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Aim: In adults 5% of intoxications are due to hydrocarbons, and the most common hydrocarbon is petroleum products. This intoxication is more

common in children. Hydrocarbon injection is not extensively addressed in the literature.

Case: This is a 21 year old girl who attempted suicide by kerosene oil injections in right upper quadrant and right lower quadrant. She was admitted to gynecologic ward with fever and dyspnea due to the dead fetus and patient toxicity she was brought to the operating room and abscess drainage and hysterotomy was done. Four days after operation she was discharged with good general condition.

Discussion: Many cases of petroleum injection as a suicidal attempt is addressed in literature, but this is a rare case of embryo toxicity and fetal death due to petroleum oil injection.

Conclusion: Fetal death may be a direct effect of petroleum oil soft tissue injection.

91. ORALVEQ: External quality assessment scheme of drugs of abuse in oral fluid. Results obtained in the first round performed in 2007

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Introduction: The Pharmacology Research Unit of IMIM-Hospital del Mar (Barcelona, Spain) in cooperation with the Department of Therapeutic Research and Medicines Evaluation of Istituto Superiore di Sanità (Rome, Italy) organized an external quality assessment scheme (ORALVEQ) to know the quality and the methodological approach applied by laboratories when analysing drugs of abuse in oral fluid. The first round of ORALVEQ was performed in February 2006. The results obtained are presented.

Methods: Three different samples (S1, S2 and S3) were sent to 21 laboratories. S1 was a blank sample and S2 and S3 were prepared by addition of known concentrations of drugs to pre-screened drug-free oral fluid. The samples were prepared with drug-free oral fluid (containing sodium azide), spiked with substances and diluted up to 50% with acidic buffer. S2 contained 6-monoacetyl morphine, morphine, cocaine and benzoylecgonine and S3 contained 3,4-methylendioxymethamphetamine and 3,4-methylendioxyamphetamine. Spiked samples were mixed, checked for homogeneity and verified by three reference laboratories before sending them to the participating laboratories. Results were evaluated from a qualitative and a quantitative point of view. The qualitative evaluation was done calculating false negative (FN) and false positive (FP) findings and the quantative evaluation was performed calculating the z-score values using the robust mean and the robust standard deviation of all participating laboratories. Dispersion (CV%) and accuracy (ERR%) of results were also measured.

Results: Only half of laboratories performed screening analysis and only half of them applied the cut-off concentrations recommended by SAMHSA. Concerning identification/quantification, all laboratories reported a quantitative result for almost all analytes and between 50% and 74% of laboratories (depending on the analyte) applied the SAMHSA recommended cut-off concentrations. GC-MS was the analytical technique most used, followed by HPLC-MS/MS. Regarding the qualitative results, no FN and 10 FP were reported by 2 laboratories in the screening analysis. In the identification, 8 FP were reported by 3 of the 21 laboratories. Concerning quantitative results, ERR% were around 10% for all analytes except for 6-monoacetyl morphine (20%) and CVs% were around 40% for all the analytes. In terms of z-score, high percentage of satisfying results (between 85 and 95%) were obtained for all the analytes.

Conclusion: Results obtained showed a global satisfactory performance of laboratories. The number of FN reported was very low and the FP were reported by a reduced number of laboratories and could be related to the interference of the resulting oral fluid to one of the immunoassay used. Finally, the scatter in the quantitative results was principally due to a few outlying values, since applying robust statistics no rejection of outliers was done.

Keywords: oral fluid, drugs of abuse, external proficiency testing

92. Quantitation of anhydroecgonine methyl ester (AEME) in oral fluid using liquid chromatography-tandem mass spectrometry

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Aims: While all forms of cocaine use are problematic, the use of crack cocaine presents a greater social problem, and has a high association with crime. The ability to specifically identify the use of crack cocaine would provide a valuable addition to drug treatment and criminal justice systems. It has been clearly established in the literature that the pyrolysis of cocaine produces a unique compound, anhydroecgonine methyl ester (AEME). What is less clear is how useful AEME is for identifying the use of crack cocaine in a variety of biological matrices. The aim of this study was to develop a sensitive LC-MS/MS method for the simultaneous analysis of cocaine, its metabolites and pyrolysis products in oral fluid. The analysis of clinical samples using this method would provide information on the concentration of AEME present in oral fluid, and determine its reliability as a marker for identifying crack cocaine use.

Methods: A total of five different analytes, EME, AEME, benzoylecgonine, cocaine and cocaethylene, were extracted using mixed mode SPE. Analysis was performed on a Varian 1200L LC-MS/MS using an Agilent Eclipse XDB-Phenyl column, and gradient elution with a mobile phase of 10 mM ammonium formate pH3/0.1% formic acid in methanol. Validation of the method involved determining the linearity, LOD, LOQ, precision, accuracy, selectivity, recovery, and ion suppression. Once the method was fully validated a total of 270 oral fluid samples from a cocaine using population were analysed to provide valuable quantitative data.

Results: The method was linear for each analyte over the range of LOD – 360 ng/mL. The LOD and LOQ for all analytes were 1 ng/mL and 2 ng/mL respectively, with the exception of AEME which was 0.5 ng/mL and 1 ng/mL. The inter-assay precision was 1.9-7.1%, and the intra-assay precision was 1.4-4.9%. The accuracy was between 0.1 and 16%, with benzoylecgonine giving the greatest % bias. The method showed good selectivity with no interfering peaks present in the chromatograms. The average extraction recoveries were as follows, EME 53.6%, AEME 88.3%, benzoylecgonine 99.1%, cocaine 72.9%, and cocaethylene 72.1%. There was no significant ion suppression for any of the analytes.

A total of 91% of the clinical samples analysed were positive for benzoylecgonine and of this 34% of these samples were positive for AEME. The highest concentration of AEME detected was 989 ng/mL, this exceeds other reported oral fluid concentrations from a drug using population. While there was no correlation between the concentrations of AEME with cocaine or any of its metabolites, it was observed that in general its presence was accompanied by significantly higher concentrations of benzoylecgonine and cocaine.

Conclusion: The LC-MS/MS method was shown to be very sensitive and reliable for the detection of cocaine, its metabolites and pyrolysis product. The clinical data shows the possibility to positively identify the use of crack cocaine using oral fluid samples although the presence of AEME with very high concentrations of cocaine and benzoylecgonine suggest a short half life.

Keywords: oral fluid, crack cocaine, LC-MS/MS

93. Nails of newborns as a means in monitoring drug exposure during pregnancy

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Introduction: According to our knowledge, nails of a newborn have been used to prove the prenatal exposure to cocaine in a single case of sudden death of a 3-month-old infant [Skopp and Pötsch, Ther Drug Monit. 1997; 19: 386-389]. In consideration of the vast consequences of drug abuse during pregnancy and to the importance of opportunely anticipating the onset of withdrawal symptoms in newborns, this project was developed to investigate the usefulness of newborn nails for monitoring *in utero* drug exposure.

Methods: Cocaine, benzoylecgonine, morphine, methadone, caffeine, nicotine, and cotinine were determined in nail samples from the first 3 months of the newborns life. Participants included 25 newborns abandoned immediately after birth (group 1) and 33 babies born at the local maternity hospital whose families were recruited on a voluntary basis (group 2). All substances were measured by a validated method that included overnight incubation in an acidic medium, solid phase extraction, and gas chromatography-mass spectrometry (detection limit: 0.025 ng/mg). Analytes were identified by the acquisition of at least 3 ions in selected ion monitoring. Moreover, analytical results were compared with mothers' self-reported habits when the information was available.

Results: Analytical data from group 1 revealed that 12 nails were positive for caffeine and 13 for both nicotine and cotinine. Six samples tested positive for both cocaine (range, median: 0.14-0.25, 0.175 ng/mg) and benzoylecgonine (0.12-0.20, 0.165 ng/mg). Nicotine and cocaine were always retrieved together with their main metabolite. Morphine was found in 4 samples (0.10-0.15, 0.125 ng/mg) and methadone was present in 5 samples (0.12-0.26, 0.170 ng/mg) that were found negative for all other compounds. Only one sample tested negative for all compounds.

In group 2 two samples tested positive for methadone (0.16, 0.17 ng/mg). No other drugs of abuse were retrieved. The mothers self-report of the use of coffee always corresponded to caffeine positivity in the newborn nails (n=6), whereas 6 samples tested positive for nicotine and/or cotinine with a self-declaring non-smoking mother. Sixteen out of the 33 samples of group 2 tested negative for all compounds.

Conclusion: Results showed that, once that a good compliance by the mothers in the collection of samples is reached, nails of the first period of life can be a very interesting indicator of *in utero* drug exposure.

Keywords: *in utero* exposure, nails, drugs of abuse.

94. Liquid chromatography-tandem mass spectrometry for ethylglucuronide and ethylsulfate in meconium: new biomarkers of gestational ethanol exposure

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Introduction: In recent years, fatty acid ethyl esters (FAEEs) in meconium emerged as reliable, direct biological markers for establishing gestational alcohol exposure. Conversely, ethyl glucuronide (EtG) and ethyl sulfate (EtS), two ethanol metabolites already detected in blood, urine and hair have been never investigated in this neonatal biological matrix. We developed a sensitive and specific LC-MS/MS method for the determination of EtG and EtS in meconium using pentadeuterated EtG as internal standard.

Methods: The analytes were initially extracted from the matrix by acetonitrile. Subsequently a solid-phase extraction with aminopropyl-silica columns was applied. Chromatography was performed in isocratic mode on a C_{18} reversed-phase column using a mobile phase containing 99% formic acid (0.1%) and 1% acetonitrile. Flow rate was set at 0.2 ml/min. Detection was performed with a triple-quadrupole mass spectrometer that monitored two specific transitions per compound (*m/z* 221 → 75 and 221 → 85 for EtG, *m/z* 125 → 97, 125 → 80 for EtS and *m/z* 226 → 75 and 226 → 85 for pentadeuterated-EtG) in the electrospray negative ionization, enhanced by post-column addition of acetonitrile (0.1 ml/min).

Results: Limit of Detection (LODs) and Lower Limit of Quantification (LLOQs) for EtG were 3 and 5 ng/g, while for EtS were 0.7 and 1 ng/g respectively. Calibration curves were linear from LLOQs to 500 ng/g with a minimum r^2> 0.999. At three concentrations spanning the linear dynamic range of the assay, mean recoveries ranged between 51.4 and 75.3% for EtG and between 71.8 and 100% for EtS. Accuracy and precision (intra and inter-day), studied at four different quality control levels, were always within the required thresholds. Matrix effects (ion suppression/enhancement) were found negligible. The fully validated method was applied to analysis of meconium in a cohort of new-borns from Reggio Emilia, Italy to assess potential fetal exposure to alcohol. Range of found concentrations in the first 60 samples ranged between 5.0 and 796.3 ng/g for EtG and between 1.0 and 65.2 ng/g for EtS.

Conclusion: The roughness and robustness of the developed LC-MS/MS method make it suitable for use as high-throughput assay in assessing fetal exposure to alcohol in risky environments. From the preliminary results, EtG and EtS in meconium seem to be a good alternative to FAEEs as biomarkers of chronic *in utero* exposure to alcohol.

Keywords: ethyl glucuronide, ethyl sulfate, meconium, LC-MS/MS

95. Novel biomarkers of in utero methamphetamine exposure in meconium

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Introduction: Identifying methamphetamine (MAMP)-exposed neonates is critical for determining maternal and neonatal toxicities, developing prevention strategies, and establishing medical and social interventions for children and their mothers. Over 15,000 infants evaluated in the largest study of prenatal MAMP use, the Infant Development, Environment, and Lifestyle (IDEAL) study showed MAMP-exposed neonates had smaller head circumference, lower birth weight, and decreased gestational age than non-MAMP-exposed children. Of immunoassay amphetamine-positive meconium IDEAL specimens, only 40.7% confirmed positive for MAMP and/or AMP. Other MAMP and AMP metabolites in meconium have not been investigated and could lead to more sensitive identification and improved medical treatment of drug-exposed infants.

Methods: A validated LC/MS/MS method simultaneously quantified MAMP, AMP and 3 potential new biomarkers, *p*-hydroxymethamphetamine (pOHMAMP), *p*-hydroxyamphetamine (pOHAMP), and norephedrine (NOREPH) in 43 MAMP positive specimens. Method validation included determination of sensitivity, selectivity, imprecision, matrix effects, accuracy, and stability. Limits of quantification (LOQ) were 12.5 ng/g for pOHAMP, NOREPH, AMP, MAMP and 8 ng/g for pOHMAMP in 1 g meconium. Imprecision was less than 14.2%, with accuracy between 79-115%.

Calibration curve coefficients of determination were greater than 0.979 and all calibrators were within ±20% of target. Extraction efficiencies ranged from 74.2 – 80.6%, and matrix effects were between 85.2 – 149.4%. Freeze-thaw and autosampler stability was greater than 85%.

Results: All specimens were positive for MAMP [mean ±SD, range (1621±1556 ng/g, 141 – 8259)] and AMP (355±351, 13 – 1837), and 86% were positive for the novel analyte, pOHMAMP (79±71, 18 – 413). Eleven of 43 (26%) also were found to contain NOREPH (26±9, 16 – 39 ng/g). Although pOHAMP was identified in 4 specimens, concentrations never exceeded the LOQ. On average, MAMP concentrations were 4 and 12 times greater than concentrations of AMP and pOHMAMP, respectively, and were significantly correlated with AMP (r=0.808, p<0.0001) and pOHMAMP (r=0.636, p<0.0001) concentrations, but not with NOREPH.

Conclusion: These data describe the disposition of MAMP and four metabolites in human meconium after methamphetamine exposure during gestation. It is possible that one of these additional analytes could be important for predicting toxicity or maternal or neonatal outcome measures and also could contribute to positive AMP/MAMP immunoassay tests.

Keywords: *In utero* methamphetamine exposure, methamphetamine meconium biomarkers; prenatal drug exposure

96. Method development and validation for analysis of methadone, heroin, cocaine and metabolites in sweat patches by solid phase extraction and GC/MS

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Aims: Advantages of sweat testing include the ease and non-invasiveness of specimen collection and a reduction in the potential for specimen adulteration. These advantages have created interest in monitoring drug use through testing in driving under the influence, drug treatment, criminal justice, and workplace drug testing programs. We have developed and validated an electron impact GC/MS method to simultaneously quantify methadone, heroin, cocaine and metabolites in sweat patches.

Methods: Blank sweat patches were moistened with artificial sweat, spiked with working calibrator and internal standard solutions (anhydroecgonine methyl ester (AEME-d_3), cocaine-d_3, acetylcodeine-d_3 and heroin-d_9), and extracted twice using a total of 9 mL of 0.5 M sodium acetate buffer (pH 4.0). A 5-mL aliquot of the combined buffered extract was applied to SPE columns (Clean Screen, UCT) preconditioned with acetonitrile, water and sodium acetate buffer. Columns were washed with water, 0.1 M acetic acid, and acetonitrile and eluted with methylene chloride: 2-propanol: ammonium hydroxide (78:20:2). After evaporation, extracts were derivatized with BSTFA/TMCS and analyzed. A separate SPE extraction using the same procedure was performed for the quantification of heroin utilizing 3 mL of the remaining buffered extract and ethyl acetate: triethylamine (98/2) as the elution solvent.

Results: Daily calibration curves for AEME, ecgonine methyl ester, cocaine, benzoylecgonine, codeine, morphine, acetylcodeine, 6-acetylmorphine, heroin (5-1000 ng/patch) and methadone (10-1000 ng/patch) all exhibited correlation coefficients >0.995. Extended calibration curves (1000-10,000 ng/patch) were constructed for methadone, cocaine, benzoylecgonine and 6-acetylmorphine by modifying injection technique from splitless to a 1:10 split injection. Within-run (N=5) and between-run (N=20) precision were calculated at six control levels (15, 150, 750, 1500, 3000 and 8000 ng/patch) with coefficients of variation of less than 6.5%. Accuracies at the same concentrations were ±11.0% of target concentrations. Similarly, mean extraction efficiencies for all controls (N=4) were in the range of 68.8%-118.5% for all analytes. Forty-seven potential interfering compounds were spiked (1000 ng/patch) into low quality control samples (15 ng/patch) to evaluate specificity, and all quantified within ±20% of target. Analyte stability was determined by subjecting quality control samples (N=5) to three freeze-thaw cycles. Accuracy was reported as ±11.9% of target for all analytes. Hydrolysis of heroin during method processing was found to be <11%.

Conclusion: We present a rugged, sensitive and specific method to simultaneously quantify methadone, heroin, cocaine and metabolites from sweat patches.

Keywords: sweat patches, methadone, drug of abuse

97. Application of LC-TOF-MS qualitative screening analysis to vitreous humour

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Introduction: Several years' experience with LC/TOFMS has proven the high performance of the technique for comprehensive screening of urine samples in post-mortem and clinical contexts. In the authors' method, accurate mass measurement is followed by automated search using a target database of exact monoisotopic masses. Entries of the large target database, representing the elemental formulae of reference drugs and their metabolites, are compared with the measured masses for protonated molecules. The success with urine matrix prompted us to widen the scope of the method to other, less common matrices. In this study, the applicability of the technique to vitreous humour analysis was investigated.

Methods: Sample preparation was based on C-4/cation exchange mixed-mode solid phase extraction. Chromatographic separation was performed with C-18 reversed phase liquid chromatography using a gradient with ammonium acetate in formic acid and acetonitrile as the mobile phase. Mass analysis was performed with a micrOTOF (Bruker Daltonics) spectrometer, applying electrospray ionization in positive mode. TargetAnalysis 1.1 (Bruker Daltonics) software performed the mass scale calibration of the data and created extracted ion chromatograms in 0.002 m/z window for the protonated molecule of each molecular formula in the list. Subsequently, the software applied peak detection and identification criteria according to mass accuracy, isotopic pattern (SigmaFit), peak area, and retention time if available, and finally created a MS Excel-based result report. The findings in vitreous humour were compared with the findings in blood and urine in order to evaluate the correctness of the results. Our current in-house database includes 815 masses comprising a wide variety of drugs, drugs of abuse, metabolites, and designer drugs.

Results: The LC-TOF-MS method was successfully applied to qualitative analysis of vitreous humour, which proved to be a representative and easy-to-handle sample for screening analysis in post-mortem cases, where urine is not available. The drugs identified included opioids, antidepressants, antipsychotics, antiepileptics, benzodiazepines, beta blockers, hypnotics, and anticoagulants. The limit of detection was determined for 70 drugs, and the median LOD-value was 23 µg/L. Ion suppression was studied by post-column infusion against blank matrix with selected compounds with retention times over the whole run time: metformin, nicotine, morphine, oxycodone, moclobemide, propranolol, and clomipramine. Ion suppression was moderate compared to urine matrix, and occurred sporadically at 0.7-1.6 min, thus disturbing only a few, early eluting compounds. The maximum suppression was 18% (morphine, RT 1.37 min).

Conclusions: This study demonstrates, for the first time, the feasibility of LC-TOF-MS in the comprehensive screening of drugs in vitreous humour. The sample preparation procedure developed for urine samples was easily adapted to vitreous humour analysis, and clean extracts with low background were obtained. Consequently, legible results reports were produced by the automated data handling software.

Keywords: LC-TOF-MS, accurate mass, screening

98. Comparison of methamphetamine concentrations in oral fluid, urine and hair of twelve drug abusers using solid-phase extraction and GC-MS

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Introduction: Methamphetamine (MA) is the most abused drug in Korea. In order to investigate the correlation of MA disposition in oral fluid, urine and hair specimens, quantitative analysis for MA and its main metabolite amphetamine (AM) was performed. Twelve drug abuser's oral fluid, urine and hair samples were used, which were submitted by the Police for drug testing.

Methods: As the preliminary test, urine and oral fluid samples were screened for MA by Fluorescence Polarization Immunoassay (TDxFLx, Abbott Co.). Besides the preliminary test, all urine samples were screened for other drugs by the automated identification system, which wasdeveloped in-house for rapid detection of drugs. Extraction for MA was performed using solid-phase extraction by RapidTrace™ (Zymark, U.S.A) after dilution with phosphate buffer or distilled water. Hair samples were finely cut (<1mm) and incubated for 20 h in 1 mL methanol containing 1% hydrochloride. Samples were evaporated and derivatized by pentafluoropropionic acid anhydride (PFPA) for oral fluids and urines and trifluoroacetic acid anhydride (TFAA) for hair samples. Quantitation of MA and AM was performed by gas-chromatography mass spectrometry (GC-MS) using selective ion monitoring (SIM) for oral fluid and hair samples and full scan mode for urine samples.

Results: Concentrations of MA and AM in twelve urine samples ranged 120.4-48414.0 ng/mL and 67.6-4238.4 ng/mL, respectively. For oral fluids, just one sample−gave negative result, and concentrations of MA and AM in eleven samples ranged 104.2-4603.3 ng/mL and 32.4−268.6 ng/mL, respectively. Urinary MA and AM concentrations were substantially higher than those in oral fluids. When compared the ratios of concentration of urinary MA to oral fluid, the ratio of MA ranged 2.5-30.5, which was very similar to those in AM, which were ranged 2.7-30.1 (n=11). In the case of hair, the concentrations of MA and AM were 2.43-98.25 ng/mg and 0.23-4.35 ng/mg, respectively in twelve samples.

Conclusion: Even though there was no information for the time of drug use, dose and the time of sample collection, we found a good correlation of the qualitative results for MA and AM between urine, oral fluid and hair. In addition, all positive results for MA have an order in their concentration by urine, oral fluid and hair with no exception.

Keywords: methamphetamine, oral fluid, urine, hair

99. Profiling of ecstasy tablets by elemental analysis with ICP-MS

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Introduction: Chemical profiling of ecstasy tablets is an important process in the control of the international trade in these products. Profiling enables identification of possible reaction pathways being used for the manufacture of drugs. This allows identification and, if necessary, regulation of the raw materials. Tablets of different seizures can be linked and commercial traffic can be identified. Additives or impurities, which are potentially dangerous for public health, can be identified.

Methods: Thirty-six different types of tablets, coming from 8 different seizures were provided to us by the police. Six mL 65% HNO_3 and 1 mL 30% H_2O_2 were added to a tablet in a 10 mL Sarstedt polypropylene tube. This was incubated for 16 h at 90°C. After cooling, the tubes were centrifuged (5 min at 1000 rpm) and the supernatant was transferred to a 50 mL Sarstedt polypropylene tube. Six mL water was added to the precipitate in the first tube. After mixing and centrifugation, the supernatant was added to the first aliquot. Distilled water was added to a volume of 50 mL. This was analysed by ICP-MS on a Perkin Elmer ELAN DRC-e in Totalquant mode. A full mass spectrum (m/z=6–238, omitting the mass ranges 16–17, 40 and 41) was obtained by full mass range scanning. A reference response table (Perkin-Elmer Total Quant III) was updated with a multielement standard solution at 10 µg/mL of metals. No DRC gases were used. The software of the instrument performs automatic corrections of isobaric interferences. The experimental conditions used for the measurements were: dwell time=50.0 ms; sweeps/reading=5; reading/replicates=1; replicates=1; time per run=61 s. The concentration of the following elements was recorded: Li, Be, B, C, N, Na, Mg, Al, Si, P, S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Kr, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, I, Xe, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Th, U. Two tablets from each type were analysed. To reduce the influence of very large peaks the square root of the normalized peak area was used for determining the Pearson correlation coefficient. The organic impurities were determined by GC-MS with the method of van Deursen et al. [Sci Justice. 2006; 46:135-152].

Results: The data for N and Na were not used for the comparison, because the software did not report them for all tablets. The following elements were not observed (<50 ng/tablet) in any of the tablets: B, Sc, Ge, Kr, Ru, Rh, Pd, Ag, Cd, In, Sb, Te, Xe, Cs, Hg, Tl, Bi, Th, Pr, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ir. Platinum was present in tablets from five of the eight seizures, suggesting its use as a catalyst in the synthesis of MDMA.

Based on our preliminary results, a Pearson correlation coefficient > 0.96 suggests that the tablets belong to a single lot. Most differences that had been observed with GC-MS profiling were also found with ICP-MS, e.g. homogeneity of seizures 4 and 7 (3 & 17 different types respectively based on colour and logo), two subgroups in seizures 5 and 8. While no differences could be observed between seizure 1 & 2 with external characteristics or GC-MS, there were clear differences with ICP-MS (Pearson correlation 0.87), suggesting that they were different batches from the same lab.

Conclusion: Our results indicate that element analysis can be used for profiling ecstasy tablets. The classification of the tablets is generally similar to that of organic impurity profiling with GC-MS, but some extra information can be obtained.

100. Δ9-Tetrahydrocannabinolic acid A - a novel marker for recent cannabis consumption?

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Introduction: Δ9-Tetrahydrocannabinolic acid A (Δ9-THCA A) is the non-psychoactive biosynthetic precursor of THC and the predominant component of the cannabinoid fraction in fresh plant material of *cannabis sativa*. Although it was assumed that Δ9-THCA A is decarboxylised quickly and completely when heated, recently Dussy et al. showed that only approximately 30% of the compound is converted to Δ9-THC during smoking and Jung et al. demonstrated that Δ9-THCA A can be detected in blood and urine samples of cannabis users. The aim of the presented pilot study was to evaluate the suitability of Δ9-THCA A as a marker of recent cannabis intake. Additionally, the metabolic pattern in man was compared to the results previously obtained in a rat model.

Methods: After isolating Δ9-THCA A from seized plant material, a pilot self experiment was designed to gain first data on the excretion profile and information about the human metabolism of Δ9-THCA A. 50 mg of the compound were dissolved in 1 mL soy bean oil and taken orally. 13 blood samples and 29 urine samples were collected over a 32 hours and 96 hours period, respectively. Qualitative and/or quantitative analyses for Δ9-THCA A, the main oxidative metabolites and their corresponding glucuronides were performed by LC-MS/MS, GC-MS and/or GC-MS/MS.

Results: Δ9-THCA A was detected in all serum samples with a maximum level of 2.5 µg/mL occurring about three hours after ingestion. About one hour after ingestion the first oxidative metabolites and their glucuronides were detectable. In urine Δ9-THCA-A glucuronide, 11-hydroxy-Δ9-THCA A and 11-nor-9-carboxy-Δ9-THCA A as well as their corresponding glucuronides were identified as the main metabolites in agreement with the results obtained in the Wistar rat model. Only traces of the parent compound were detected. Relatively low signals for Δ9-THCA A glucuronide were detected in the urine sample collected 24 hours after ingestion in comparison to the urine collected in the early elimination phase.

Conclusions: The relative oral bioavailability of THCA A seems to be substantially higher than it is for THC, suggesting a considerably less pronounced first pass effect. In contrast to THC, which is subject to extensive distribution and redistribution processes, resulting in an extremely long terminal elimination half-life, Δ9-THCA A seems not to be distributed to the same extent. This is suggested by the unexpected high THCA A serum levels and the concentration profiles of the metabolites. Therefore it can be assumed that Δ9-THCA A and its metabolites could be valuable markers for recent cannabis intake, especially in cases of frequent drug abuse, since an accumulation as it is observed in the case of THC seems to be unlikely. Further research is scheduled to gain reliable data e.g. on the elimination half-life and the detection windows after single and multiple cannabis intake. It comprises controlled studies with more participants and additional inhalative/intravenous application.

Keywords: Δ9-Tetrahydrocannabinolic acid A, cannabis marker, metabolism

101. Distinguishing new cannabis exposure in occasional users by time intervals between positive urine specimens

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Introduction: Distinguishing new cannabis use from residual cannabinoid excretion is important in judicial and treatment urinalysis programs. Huestis and Cone reported that for pairs of urine specimens collected more than 24 h apart from occasional users who smoked a cannabis cigarette, the ratio of creatinine normalized 9-carboxy-delta-9-tetrahydrocannabinol concentrations (normalized THCCOOH=THCCOOH/creatinine) in the second specimen divided by that in the previously collected specimen (U2/U1) could be useful for this purpose [J Anal Toxicol, 1998; 22: 445-454]. When all specimens with THCCOOH ≥15 ng/mL were considered and new use was designated for urine pairs with U2/U1 ≥0.5, accuracy, false positives and false negatives were 85.4%, 5.6% and 7.4%, respectively. For a suggested conservative ratio, U2/U1 ≥1.5, the performance values were respectively 74.2%, 0.1% and 24%. Since the expected ratio is a function of the time between specimens, we re-examined U2/U1 for specimens, donated only during the drug elimination phase, by collection intervals that were 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-166 h.

Methods: Following informed consent, six occasional cannabis users residing on a closed, research unit each smoked a placebo, 1.75% and 3.55% THC cigarette with a 7-day washout period between administrations in a randomized design. Every urine specimen was individually collected over 21 days (N=955) and analyzed by GC/MS for total THCCOOH and by picric acid/spectrophotometry for creatinine.

Results: The range of times to peak normalized THCCOOH in urine were 6 to 11.3 h after smoking; for this new analysis only urine pairs after peak were considered (n=11,416). Data were further categorized into a group with THCCOOH ≥15 ng/mL, a common administrative cutoff concentration in urine drug testing programs for designating a positive result, and a group with THCCOOH ≥6 ng/mL, the limit of quantification for many laboratories. Minimum, maximum, median and number of U2/U1 ratios for each day between urine collections and number of ratios exceeding 0.5 and 1.5 are included below:

	0-24h	24-48h	48-72h	72-96h	96-120h	120-144h	144-166h
THCCOOH ≥15 ng/mL							
n	331	179	62	17	9	0	0
Min U2/U1	0.213	0.073	0.038	0.038	0.054	---	---
Max U2/U1	1.826	1.045	0.529	0.250	0.116	---	---
Median U2/U1	0.672	0.323	0.178	0.165	0.074	---	---
n U2/U1 ≥0.5	249	33	1	0	0	---	---
n U2/U1 ≥1.5	4	0	0	0	0	---	---
THCCOOH ≥6 ng/mL							
n	962	687	400	209	120	49	17
Min U2/U1	0.213	0.073	0.024	0.023	0.026	0.025	0.020
Max U2/U1	2.130	1.603	1.476	1.631	0.481	0.175	0.071
Median U2/U1	0.725	0.419	0.262	0.170	0.102	0.060	0.036

Conclusion: For urine specimens collected in the drug elimination phase with THCCOOH ≥15 ng/mL, collections separated by more than 24, 48, 72 and 96 h all U2/U1 were <1.1, 0.6, 0.3 and 0.2, respectively. These data provide additional empirical evidence for the interpretation of new cannabis use from paired urine specimens.

Keywords: urine cannabinoids, new cannabis use, residual excretion

POSTER PRESENTATIONS

P1. Chiral analysis of R/S-methadone and its main metabolite R/S-EDDP in postmortem blood by liquid chromatography-mass spectrometry

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Aim: A chiral LC-MS/MS method was developed for the measurement of *R*,*S*-methadone and *R*,*S*-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium (*R*/S-EDDP) enantiomers in postmortem blood.

Methods: 0.100 g blood was mixed with water and aqueous D_3-methadone and D_3-EDDP solutions. After addition of NaOH, extraction with butyl acetate was carried out. Chromatographic separation was performed on a chiral-AGP analytical column (100 mm x 4.0 mm i.d., 5 µm) with a mobile phase of acetonitrile-ammonium acetate buffer (10 mM, pH 7.0, v/v 22:78). A Quattromicro (tandem quadrupole mass spectrometer, Waters) was coupled to the HPLC system (Agilent 1100) and operated in the positive ion mode with an electrospray (ESI) source. Multiple reaction monitoring (MRM) was used with two transitions for each compound.

Results: The calibration curves were linear over the range from 0.001 to 2.5 mg/kg blood per enantiomer for all compounds. The LoD was 0.001 mg/kg, while the LoQ was 0.003 mg/kg. The precision (CV%) was below 6.5% for all compounds and the accuracy between 88-104%. *R*- and *S*-methadone were measured in femoral blood from 10 postmortem cases. The *R*-methadone concentrations extended from 0.006 to 1.235 mg/kg with a median of 0.41 mg/kg, and the *S*-methadone concentrations ranged from 0 to 0.794 mg/kg (median 0.33 mg/kg). The median *R*/*S*-ratio was 1.46 with a range from 1.00 to 2.62. *R*-EDDP concentrations extended from 0.008 to 0.132 with a median of 0.055 mg/kg, and *S*-EDDP were in the interval from 0.005 to 0.161 with a median of 0.073 mg/kg. The *R*/*S*-ratios of EDDP were from 0.60 to 1.50 with a median of 0.90.

Conclusion: A sensitive, specific and simple method for measurement in postmortem blood was established and found to be very robust.
Keywords: *R/S*-methadone, postmortem blood, chiral LC-MS/MS

P2. Enantiomeric determination of tramadol and o-desmethyltramadol by GC/MS in urine

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Introduction: Tramadol (T) hydrochloride (1RS, 2RS)-2-[(dimethylamine) methyl-1-(3-methoxyphenyl)-cyclohexanol HCl]) is a centraly acting analgetic drug presenting analgetic efficacy and potency that ranges between weak opioids and morphine. T is metabolized in the liver mainly to O-desmethyltramadol (ODT), N-desmethyltramadol (NDT) and N,O-desmethyltramadol (NODT). Of these three metabolites, only ODT is pharmacologically active. (+)-T is 10-fold more potent than (-)-T and the affinity of (+)-ODT for the μ-opioid receptor is 200 times greater than that of T.

Method: A gas chromatographic-mass spectrometric assay with positive-ion EI ionization (GC/MS) is presented for quantification (selected ion monitoring) of T and ODT in urine after solid phase extraction (SPE). The enantiomeric separation was achieved on a Restek Rt-βDEXcst column containing alkylated beta-cyclodextrins as chiral selector.

Results: Acceptable assay precision (<3.0% C.V. (T), <10% C.V. (ODT)) were observed in the linear range 0.1 μg/mL-20 μg/mL. The method was successfully applied to the determination of the enantiomers of T and ODT in urine samples from 10 pediatric patients treated with T. Mean urine concentrations were determined as follows: 1.67, 1.69, 0.88 and 0.79 μg/mL for (+)-T, (-)-T, (+)-ODT and (-)-ODT, respectively.

Conclusion: This assay may be used for further pharmacokinetic studies in patients treated with tramadol.

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Keywords: tramadol, o-desmethyltramadol, enantiomers, GC/MS

P3. Quantification of 24 benzodiazepines and 2 analogues, by Ultra-Performance Liquid Chromatography- tandem mass spectrometry, in whole blood

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Introduction: Widely prescribed, benzodiazepines and analogues are among the most detected drugs in biological fluids. Several methods for the determination of benzodiazepines and analogues, in biological samples, by HPLC with mass spectrometry or tandem-mass spectrometry have been described. However, these methods have a run time more than 20 min. The aim of this study was to develop an UPLC-MS/MS method, adapted to routine applications, allowing rapid, specific and sensitive determination of benzodiazepines and two analogues for clinical and forensic toxicology within 8 min.

Methods: 1 mL of whole blood was spiked with 50 μl of internal standard (1 μg/mL for Estazolam-d5 and 500ng/mL for Prazepam) and 500 μl of carbonate buffer pH=9.7 (0,5M). Six mililiters of hexane/dichloromethane (50:50, v/v) were added to the mixture. The residue was reconstituted with 100μl of methanol and 2 μl were injected into the UPLC unit. Separations were performed at 40°C on a Acquity UPLC® BHE C18 50*2.1mm; 1.7 μm (Waters, Milford MA, USA). The mobile phase consisted of a gradient of acetonitrile - ammonium formate buffer (4mmol/L pH 3.2). Acetonitrile was increased linearly for 20% to 50% over 5.20 minute and then set at 50% for 1.3 minute. The system was then set at the initial conditions in 10 seconds and was re-equilibrated during 1.4 minutes before the next injection. The total run time of the analysis was 8 minutes at a flow rate of 0.4 mL/min. A Acquity TQD® detector (Waters, Milford MA, USA) with electrospray ionisation (ESI) in positive ion mode was used for detection. The mass spectrometer was operated in the multiple-reaction monitoring (MRM) mode.

Results: Two groups were distinguished. The present method showed a good intra-assay precision and accuracy for all compounds, with CV values lower than 13.7% and bias lower than 15.9%. The extraction recoveries were between 62 and 89% except for 7-Aminoflunitrazepam, Lorazepam and Nordiazepam. The LOQ (1 ng/mL or 5 ng/mL, depending of the drugs) makes our method convenient for analysis of benzodiazepines in case of unknown cause of death but also in cases of chemical abuse.

Conclusion: The UPLC-MS/MS method described here allows the rapid, sensitive and specific identification and quantification of 24 benzodiazepines and 2 analogues within 8 minutes. This method is suitable for the routine determination of benzodiazepines in forensic investigations.

Keywords: benzodiazepines, UPLC, mass spectrometry

P4. Fast, selective and sensitive method for the simultaneous determination of Δ9-tetrahydrocannabinol, 11-hydroxy- Δ9-tetrahydrocannabinol, 11-nor-9-carboxy- Δ9-tetrahydrocannabinol, cannabinol and cannabidiol in blood/serum and urine by GC-MS/MS

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Introduction: A method is introduced covering the routinely detected cannabinoids Δ9-tetrahydrocannabinol (THC), its two oxidative metabolites 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) as well as Cannabinol (CBN) and Cannabidiol (CBD). The aim was to substantially reduce the total run time for the routine samples investigated in the context of DUID cases for cannabinoids without loss of sensitivity or specifity.

Methods: Sample preparation consists of a SPE with a C18 phase (Chromabond C18, 500 mg, 3 mL, M&N), washing with 0.1 M acetic acid and acetonitrile:water (70:30, v:v), elution with acetonitrile and derivatisation with MSTFA. Internal standard for quantification were the corresponding trideuterated compounds, for cannabinol and cannabidiol d_3-11-nor-9-carboxy- Δ9-tetrahydrocannabinol was used. Three transitions per analyte were recorded after optimization of the collision energy for each transition.

Results: Limits of detection ranged from 0.09 to 0.58 ng/mL, limits of quantification from 0.20 to 2.15 ng/mL with excellent linearity up to 20 ng/mL for THC, 11-OH-THC, CBN and CBD and 100 ng/mL for THC-COOH.

Conclusions: The method proved to be suitable for routine cannabinoid analysis. Using a GC temperature gradient resulting in a GC run time of only 6.5 minutes a very high specificity was achieved.

Keywords: GC-MS/MS, cannabinoid analysis, fast method

P5. Correction for drug loss during storage: decomposition of diazepam and temazepam in whole blood under different storage conditions

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Introduction: Loss of analyte from biological samples during the post-mortem interval or during storage has potentially serious implications in forensic toxicology, and it would be of value to make corrections for these losses. This initial study evaluated one approach to this problem based on

measurement of the main hydrolysis decomposition product of diazepam and temazepam, 2-methylamino-5-chlorobenzophenone (MACB). Previous studies have reported on the instability of diazepam and temazepam under different storage conditions. The aims of this study were to determine MACB in whole blood as an indicator of the extent of analyte degradation as well to investigate the effects of temperature and pH on the stability of diazepam and temazepam for one month.

Method: Blank blood was spiked with diazepam and temazepam to produce final concentrations of 1000 ng/mL of each. It is known from previous work that these drugs are stable for long periods of time, therefore, for the purposes of this study, degradation of the drugs was accelerated by storing aliquots of blood at high temperature (80°C) and under acidic (pH 2) and basic (pH 12) conditions at room temperature for one month. The samples were analyzed in duplicate at days 1, 2, 4, 7, 14 and 30. Samples were extracted by solid phase extraction and extracts were analysed by liquid chromatography-tandem mass spectrometry.

Results: Recoveries of all analytes were between 85-102%. The linear correlation coefficients for all three analytes were better than 0.99. Limits of detection and lower limits of quantitation were 0.1-2 ng/mL and 0.4-7 ng/mL respectively. Intra-day and inter-day precisions were found to be 4-17% and 4-18% respectively. After one month, the diazepam concentration at 80°C, pH 2 and pH 12 had decreased from the original concentration by 30, 25 and 49% respectively whereas temazepam decreased by 100%, 77% and 100% respectively. Under all sets of conditions the MACB concentration was observed to increase as degradation of the drugs proceeded and it could be detected when the parent drugs concentration were completely disappeared. However, MACB itself was found to be further degraded under some of the conditions used. Diazepam and temazepam are more sensitive to alkaline pH than to acidic pH or high temperature. Diazepam was noted to be more stable than temazepam.

Conclusion: Degradation of diazepam and temazepam in the blood under different conditions will produce MACB as the main hydrolysis product. MACB can be reliably detected in whole blood and can be used to correct for losses of diazepam and temazepam and to indicate the original diazepam concentration. MACB can also be used to confirm the presence of these drugs in samples, especially when the drug has decomposed due to poor or prolonged storage conditions.

Keywords: benzodiazepines, degradation, whole blood, MACB

P6. Comparison of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) for drugs relevant to forensic toxicology

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Introduction: The use of LC-MS and LC-MS/MS techniques in forensic toxicology is increasing due to the sensitivity and selectivity of modern instruments. In method development, one of the first steps is the choice of the ionization technique. APCI and ESI are mainly used in forensic analyses. The aim of this study was to determine the sensitivity of APCI and ESI using a TurboV® source on an Applied Biosystems 3200 Q-Trap mass spectrometer. The sensitivity and linearity of the different ion sources was compared for the most relevant drugs encountered in forensic toxicology.

Methods: Stock solutions of 107 analytes relevant to forensic toxicology were diluted to 1000 µg/L in 50 mmol ammonium formate (pH 3.5). Two MRM transitions per analyte were optimized using Analyst® software in quantitative optimization mode. Analytes were divided into 4 groups: common drugs of abuse (30 drugs), antipsychotics (27 drugs), antidepressants (16 drugs), and miscellaneous (34 drugs). The mixtures were separated on a Hypersil® C18 column and gradient elution using an Agilent 1200 HPLC. The analytes were detected using an Applied Biosystems 3200 Q-Trap mass spectrometer in MRM mode. The linearity was checked for each analyte over a range of 0.1 ng on column up to 100 ng on-column. Signal to noise ratios at concentrations of 0.25 ng and 2.5 ng on column and were determined using peak to peak comparison (Analyst® software).

Results: ESI showed a significant higher background noise (approx. ten times) than APCI. However, for most of the analytes both ionization techniques were comparable as the signals in ESI were also approximately ten times higher. For most analytes, electrospray ionization showed slightly better signal to noise ratio. Some analytes (e.g. clonazepam, codeine, THC) showed a significantly better signal to noise ratio and lower limit of detection in electrospray mode. Clonazepam and THC-COOH had a signal to noise ratio at 2.5 ng on column of 5.5 and 16 respectively in APCI. In ESI mode, the signal to noise ratios for clonazepam and THC-COOH were 114 and 58 respectively. ESI showed for most analytes a quadratic response, whereas APCI showed a linear response over 4 orders of magnitude from 0.1 ng on column up to 100 ng on-column.

Conclusions: The ion source coupled to AB 3200 instruments is more sensitive but shows a quadratic response using the electrospray probe. APCI produces a linear response over a wide concentration range, but with significant lower sensitivity for some analytes (e.g. clonazepam, codeine, THC). Based on this research dedicated ESI methodology was employed for screening of forensic toxicology specimens.

P7. The accuracy profile: a powerful tool in forensic toxicology

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Introduction: In forensic toxicology, analytical data are presented in court and might be not contested by experts in the field. Because of this, the quality of analytical data takes a considerable importance. Therefore, all analytical methods require a complete validation after their development. A part of the validation consist to use the accuracy profile which shows in a single graph all the statistical data, such as the limit of quantification (LOQ) and the confidence intervals, and allow an estimation of the uncertainty measurement.

Methods: First, the accuracy profile was used to choice between a monolith and a RAM support for the extraction of benzodiazepines in whole blood. The accuracy profile was constructed for each compound tested which both supports. Secondly, by the development of a method to analyse THC and its metabolites in whole blood, a comparison was realized between the simple linear regression model based on the least square method and the weighted linear regression model with a weight equal to 1/x and also with a weight equal to $1/x^2$.

Results: The acceptance limits were fixed at ±30% according to the requirements generally admitted in forensic toxicology. Accuracy profiles for each benzodiazepines with both supports tested are shown. For the monolith sorbent, the LOQ of nordiazepam, oxazepam, desalkylflurazepam, flurazepam, clonazepam and lorazepam were fixed at the initial lowest values in the calibration ranges. For desmethylflurazepam, the LOQ had to be increased from 2.5 µg/L to 5 µg/L for staying within our defined criteria. For the RAM sorbent, only clonazepam and lorazepam are located between the upper and lower limits. For flurazepam and oxazepam, the LOQ was fixed at 5 and 15 µg/L respectively, to stay within the acceptance range.

The simulation of the different regression models shows the variation of the statistical data on the accuracy profile graphic. In order to fulfil to the criteria admitted in forensic toxicology, the best regression model was chosen and then a LOQ at 0.5, 0.5 and 2.5 ng /mL for THC, THC-OH and THC-COOH respectively, was fixed.

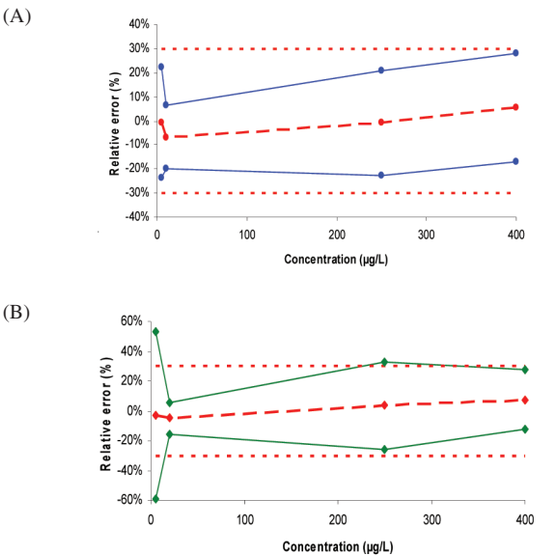

Figure 1. Accuracy profile obtained for midazolam after extraction with the monolith sorbent (A) and the RAM sorbent (B).

Conclusion: The accuracy profile is a powerful tool for toxicologist, either for choosing the best sorbent for column during a method development, either for determining validation data like the LOQ inside fixed criteria.
Keywords: accuracy profile, validation, limit of quantification

P8. Evaluation of a UPLC-TOF method using both protonated molecular ions and their fragmentation patterns for urine drug screening in hospitals

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Introduction: Since its introduction in 1992, the REMEDi™ HS Drug Profiling System (DPS) (Bio-Rad) has been used in laboratories worldwide, providing automated analysis for basic drugs. For the last 10 years, this system has been used in our 4 hospitals in Hong Kong, to complement our other screening techniques in the routine analysis of urine. Currently we handle a combined total of ~13,500 specimens/year. The decision of Bio-Rad to discontinue support of the DPS has necessitated its replacement with an alternative screening technique. In collaboration with laboratories in Denmark and the UK, we have developed a method based on UPLC-TOF mass spectrometry. We present this method in addition to a preliminary evaluation of both the transferability of the technique between our laboratories and its performance with authentic samples in comparison to REMEDI™.
Methods: Analytes were separated by UPLC (Waters), using an ACQUITY HSS C_{18} column (2.1 x 100 mm, 1.8µ) and analysed using a LCT Premier XE (Waters). To evaluate the transferability of the method between all laboratories, 30 drugs (ranging in molecular weight and chromatographic retention) were selected and used by each laboratory to prepare reference libraries based on retention time (RT) and spectral data. Spectra were acquired using two different voltages within the source region. A low voltage (V1 spectra) enabled exact mass measurement of the protonated molecular species. The higher voltage (V2 spectra) was used to generate fragments in-source (collision-induced dissociation; CID) for additional confirmatory purposes. To evaluate the utility of the developed method, 23 authentic urine specimens were analysed by UPLC-TOF and processed using ChromaLynx™. This fully automated software uses deconvolution to locate components, to compare the underlying spectral data with that in a reference library and finally to determine the match factor (MF).
Results: The transferability of the method was assessed. Retention times were demonstrated to be highly reproducible between laboratories, with an average deviation of 1.3% (from the mean RT for each of the 30 drugs).ChromaLynx™ calculated the spectra match for V1 and V2 data in comparison to both the in-house library and the libraries prepared using the collaborators' instrumentation. The deviation of MF against the average was acceptable at <9%, indicating the spectra were reproducible between instruments. Data obtained from the 23 authentic specimens were searched against an expanded drug library (237 drugs/metabolites) and the findings compared to those obtained with REMEDI™. A total of 87 compounds were detected. All analytes found by the DPS were identified using UPLC-TOF. However, an enhanced detection was observed with the latter *i.e.* additional 21 positives, involving 15 drugs including zolpidem, chlorpheniramine, ketamine, chlorpromazine, methadone, sulpiride, metoclopramide, zopiclone.
Conclusions: We have developed a screening method based on UPLC-TOF. Identification is achieved by comparison of spectral data and retention time to a prepared library. Exact mass measurement allows the prediction of probable elemental formulae; in combination with fragmentation data, this adds confidence to identification. The method appears to be a suitable alternative to our current REMEDi™ system for the screening of urine samples.
Keywords: screening, LC-TOF, REMEDi™

P9. Quantitative analysis of multiple psychotherapeutic drugs in human serum using UPLC-MS/MS

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Aim: To develop and validate a simple UPLC-MS/MS method for the simultaneous quantitation of multiple psychotherapeutic drugs in human serum for use within a clinical or forensic setting.
Methods: Quantitative analysis was performed on thirteen psychotherapeutic drugs; amitriptyline, clomipramine, citalopram, doxepin, fluoxetine, fluvoxamine, imipramine, paroxetine, protriptyline, sertraline, trazodone, trimipramine, venlafaxine and four drug metabolites; desipramine, norclomipramine, norfluoxetine and nortriptyline with three internal standards; clomipramine-D3, doxepin-D3 and imipramine-D3. Human serum samples (100 µL) were prepared by a simple protein precipitation (1:3) using acetonitrile. Chromatography was performed using a Waters ACQUITY UPLC system; analytes were separated on a Waters ACQUITY UPLC BEH C18 (2.1 x 100 mm, 1.7 µm) column using a gradient elution over 8 minutes with a mixture of 5 mM ammonium acetate containing 0.05% formic acid in water (A) and acetonitrile (B). A Quattro Premier XE tandem mass spectrometer was used for analysis with electrospray ionisation in positive mode (ESI+). Two MRM transitions were used for each compound and each transition was optimized to achieve maximum sensitivity.
Results: For all compounds, responses were linear over the investigated range (1–200 µg/L). Intra-assay precision and accuracy were good with CV's for spiked QC samples <15% and >88%, respectively. The use of a simple protein precipitation was demonstrated to be very efficient and gave reproducible extraction recoveries >92% for all analytes. The cut-off used for all compounds was 2 µg/L with limits of detection ranging from 0.1 to 1.0 µg/L. The matrix effects were assessed by spiking blank extracted patient samples (n=6) with all compounds and comparing the responses against the equivalent concentration solvent standard solution. The effects were found to

be minimal with the norclomipramine response being most affected (+10%). All compounds were shown to be stable in extracted samples over 12 hours. The method was applied to the analysis of clinical patient samples (*n*=23) which were previously analysed by an established validated HPLC/UV method; all samples showed good agreement.

Conclusion: This method provides simple, sensitive and robust solution for the quantitation of multiple psychotherapeutic drugs in human serum. When the developed UPLC-MS/MS method is compared to the established validated HPLC/UV method, the developed method has a much simplified sample preparation and a 2.5 fold reduction in chromatographic run time.

Keywords: psychotherapeutics, UPLC, serum

P10. Identification of metabolites by LC-MS-TOF demonstrated at the example of diphenhydramine

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Introduction: Identification of metabolites in biological samples is often a difficult task if no reference standards are available. For a study of drug metabolism LC-MS-TOF systems of two producers (Agilent and Waters) were used. The advantages of TOF analysis over earlier studies using HPLC-DAD are demonstrated with diphenhydramine (DPH) as an example.

Methods: Clinical and post-mortem blood and eventually liver samples were liquid/Liquid extracted with dichloromethane. Chromatographic conditions were: a) Agilent TOF for accurate mass determination: An Agilent 1100 series HPLC was coupled to the ESI-MS-TOF of the same producer. The HPLC solvents were water and acetonitrile, both with addition of 0.01% formic acid. A linear gradient was run with a C18 endcapped reverse-phase column with 2 µm particles (Agilent). b) Waters TOF for accurate mass determination and fragmentation: A LCT Premier and an Acquity UPLC system were combined. The HPLC solvents were pure water and methanol, both with addition of 0.01% formic acid. A linear gradient was run with a C18 T3 UPLC column with 1.8 µm particles (Waters Acquity). c) The HPLC-DAD measurements were performed using a Shimadzu device with an SPD-M10Avp detector, a Lichrospher RP8ec column (5 µm, 250 x 4.0 mm) with acetonitrile/phosphate buffer pH 2.3 (37:63, v/v) as mobile phase.

Results: Samples of 35 cases of DPH intoxication were analyzed with the 3 methods described above. With HPLC-DAD up to 9 possible metabolites due to spectral analysis and reproducible retention time could be detected. Only desmethyl-DPH and diphenylmethoxyacetic acid were identified using reference standards, while 2 other metabolites (didesmethyl- and N-oxide-) were characterized by retention time shift. With accurate mass determination and less than 5 ppm mass deviation, 9 metabolites were found. Two of them exhibited the same accurate mass (271.1572289 amu) and could only be distinguished by retention time (OH- or N-oxide). Without fragmentation only the sum formula of the metabolite but no structural information is available and therefore no absolute identification of the unknown metabolites is possible. In principle with the Agilent TOF, fragmentation can be performed in a separate LC run, but was not tested in our experiments. With the Waters TOF analysis accurate mass and fragmentation can be achieved in one run and the data can be correlated. In this case, structural information by fragment pattern is available and allows real identification of metabolites (desmethyl-DPH, didesmethyl-DPH, diphenylmethoxyaceticacid, DPH-N-oxide, hydroxyl-DPH, dihydroxy-DPH, hydroxymethoxy-DPH, N-acetyl-DPH, OH-N-acetyl-DPH). Metabolites with the same accurate mass can also be distinguished like DPH-N-oxide ($M+H^+$: 272.1635; 167.0842) and OH- DPH ($M+H^+$: 272.1635; 183.0803).

Conclusion: In all 3 methods retention time is a important parameter for metabolite identification. With HPLC-DAD a specific identification of metabolites is difficult, if they have very similar spectra or extremely different spectra compared to the parent compound and if no standards are available. Already with TOF analysis without fragmentation (Agilent) metabolites can be distinguished by accurate molar mass. With exact mass determination and fragmentation (Waters) in combination with software for metabolite identification also metabolites with the same accurate mass can be distinguished. Nevertheless, also the UV spectrum contributes to metabolite identification in the combined use of both techniques.

Keywords: LC-MS-TOF, metabolism, diphenhydramine

P11. Simultaneous determination of amitraz and its metabolite in human serum by monolithic silica spin column extraction and liquid chromatography–mass spectrometry

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Introduction: Amitraz (*N*′-2,4-(dimethylphenyl)-*N*-[(2,4-dimethylphenyl)imino] methyl-*N*-methyl-methanimidamide) is a formamidine derivative widely used for the control of ticks and mange mites in animals. In the body, it is metabolized to *N*-2,4-(dimethylphenyl)-*N'*-methylformamidine. Amitraz is associated with a number of side effects, the commonest being transient sedation or lethargy. Other side effects are bradycardia, hypothermia, hypotension, polyuria, vomiting, and hyperglycemia. Although chromatographic methods for the analysis of amitraz in plasma have been described in several previous publications, to the best of our knowledge, there has been no method reported in the literature for the simultaneous determination of amitraz and its metabolite using liquid chromatography–mass spectrometry (LC–MS). Here, a simple, rapid, sensitive, and specific LC–MS method was developed and validated for the quantification of amitraz and its metabolite in human serum.

Method: Both the compounds were extracted using monolithic silica spin columns with acetonitrile. The chromatographic separation was performed on a reverse-phase C_{18} column with a mobile phase of 10 mM ammonium formate–acetonitrile. The protonated analyte was quantitated in positive ionization by mass spectrometry.

Results: Using monolithic spin column extraction in conjunction with LC–MS analysis, amitraz and its metabolite were separated within 20 min. The method was validated over the concentration range of 25–1000 ng/mL for amitraz and its metabolite in human serum. For both compounds, the limit of detection was 5 ng/mL. Both the analytes were found to be stable in human serum for 30 days when stored at −30°C. The method was applied to serum samples taken from an attempted suicide patient, and only small volumes of serum were required for the simultaneous determination of these compounds.

Conclusion: A simple and convenient extraction procedure makes this method more feasible for amitraz and its metabolite. It is expected that this method could also be applied in clinical and toxicological studies.

Keywords: amitraz, monolithic silica spin column, liquid chromatography–mass

P12. Analysis of Δ9-THC and its metabolites 11-OH-Δ9-THC and THC-COOH in blood by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) and its application to medico-legal investigations

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Introduction: Polish legal regulations, especially those concerning road traffic safety mean that forensic toxicologists are required to perform qualitative and quantitative analysis of active ingredients of Cannabis, i.e. Δ^9-tetrahydrocannabinol (Δ^9-THC) and its metabolites in biological specimens.

Analytical criteria regarding determination of these compounds in blood are specified in the Decree of the Minister of Health (Journal of Laws 2003, no.116, item 1106, and Journal of Laws 2004, no. 52, item 524).

Methods: In this study the authors presented application of their procedure for determination of Δ^9-tetrahydrocannabinol (Δ^9-THC), as well as its two metabolites, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinolic (THC-COOH) acid, in ante-mortem and post-mortem blood specimens after solid phase extraction (SPE) by means of gas chromatography coupled to tandem mass spectrometry in electron impact mode (GC-EI-MS/MS). Method validation was performed according to ISO 17025:2005.

Results: The limit of detection (LOD) and limit of quantification (LOQ) were 1 ng/mL for Δ^9-THC and 11-OH-Δ^9-THC, and 5 ng/mL for THC-COOH. The linearity of the method (LOL) was within the 1–50 ng/mL range for 9-THC and 11-OH-THC, and 5–100 ng/mL for THC-COOH. Inter-assay and intra-as say precision and relative error values were in the range of 6.8–12.3 and 7.0–15.5%, respectively. The analytes' extraction recoveries were in the range of 68.7–84.1%. The developed method is illustrated by two examples of analyses of blood specimens (collected *ante-mortem* and *post-mortem*) from toxicological practice. The results are shown in Table 1.

Table 1.

Case description	Δ^9-THC	11-OH-Δ^9-THC	THC-COOH
	Concentration (ng/mL)		
A 30 years old male caused a road accident.	3.6	1.0	22.2
A 17 years old male died due to an overdose of amphetamines.	7.8	12.9	104

Conclusion: The presented analytical procedure provides specific and accurate quantification results for cannabinoids in blood in the concentration ranges found in cases of Cannabis consumption. This method enables reduction of biological matrix interference, especially that originating from post-mortem blood samples, which contain putrefaction products of decay and autolytical processes. Such an analytical solution makes the method more sensitive and increases the reliability of the results of toxicological analysis. Improving of sensitivity and selectivity as a result of MS/MS application play a great role in the analysis of blood samples collected from drivers for the presence of substances acting similarly to alcohol, which are usually present in blood in low concentrations.

Keywords: cannabinoids, blood, GC-MS/MS

P13. Rapid determination of anticoagulants and rodenticides in blood by UPLC-MS/MS

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Aim: A rapid method was developed for simultaneous determination of seven rodenticides (brodifacoum, bromadiolone, chlorophacinone, coumachlor, coumatetralyl, difethialone and diphacinone) and three anticoagulants, used in medical practice, (acenocoumarol, fluindione and warfarin) in whole blood using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS).

Methods: Whole blood samples (0.2 mL) were deproteinized by acetonitrile (0.8 mL) after addition of 200 ng prazepam as internal standard (IS). The organic phase was evaporated and the dry extracted resuspensed in 50 µL of the UPLC mobile phase, from which 10 µL were injected onto the column. The analytes were separated on an Acquity UPLC C18 column (Waters), 1.7 µm (100 x 2.1 mm, i.d.) using gradient elution with acetonitrile/methanol (50/50, v/v) and 2mM ammonium formate buffer, pH 6.0, at a flow rate of 0.5 mL/min. The duration of analysis was 5.5 min. The detection was performed with a Quattro Premier™ XE (Waters Micromass) tandem mass spectrometer set in switching positive/negative electrospray mode and multiple reaction monitoring (MRM) mode.

Results: Under these UPLC-MS/MS conditions, limits of detection (LODs) and limits of quantification (LOQs) ranged from 0.05 to 2 ng/mL and from 0.1 to 5 ng/mL, respectively, for the 10 compounds tested. Linearity was established from LOQs to 1000 ng/mL (excepted for coumatetralyl (500 ng/mL), bromadiolone and brodifacoum (2000 ng/mL)) with coefficient correlation always greater than 0.99. Recoveries, tested at three concentatrations (n=6), ranged from 49.4% to 95.5%. Intra- and inter-day precision were less than 15% and accuracy ranged from 87 to 116% for all analytes. Ion suppression/enhancement was also evaluated and was lower than 10%. As an illustration, this method was employed for the screening of anticoagulants in the blood sample of a 25 years old male admitted to ICU with a bleeding syndrome and suspected of occult ingestion of rodenticides. Chlorophacinone could be detected in the sample tested at the concentration of 1360 ng/mL.

Conclusion: The UPLC-MS/MS method herewith described was found sensitive, specific and rapid for the simultaneous quantification of anticoagulants and rodenticides in whole blood. Under these respects it could be useful to both clinical and forensic toxicology.

Keywords: anticoagulants, rodenticides, UPLC-MS/MS

P14. An ONLINE DAT® Immunoassay for the detection of amphetamine, methamphetamine and designer amphetamines in urine

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Introduction: A new assay is in development for the detection of amphetamine and related compounds in urine on automated clinical analyzers*.

Method: The liquid, homogeneous assays utilize the KIMS technology (Kinetic Interaction of Microparticles in Solution), where multiple monoclonal antibodies are covalently linked to carboxy-modified polystyrene microparticles, with drug conjugates in solution. The assay utilizes cutoff concentrations of 300, 500 and 1000 ng/mL.

Results: Studies summarized herein evaluate the 500 ng/mL cutoff (comparable results obtained for the 300 and 1000 ng/mL cutoff). When run in semi-quantitative mode on Roche/Hitachi 917 analyzers, control recovery at 250, 375, 500, 625 and 1000 ng/mL showed a recovery of 266 (106%), 387 ng/mL (103%), 511 ng/mL (102%), 648 ng/mL (104%) and 1042 (104%) with intra-assay %CV precision values of 3.6%, 3.1%, 3.2%, 2.9% and 2.9%, respectively. The interassay precision of the same levels ranged from 3.3 to 4.4%. High cross-reactivity to designer drugs is observed (d-Methamphetamine is ≥90%, d-Amphetamine is 75-125%, BDB is ≥30% and MBDB, MDEA, MDA and MDMA are ≥50%). Cross-reactivity to over-the-counter medications such as l-ephedrine and d-pseudoephedrine is ≤1%. Studies show the immunoassay displays a low background and that in the screening of samples that challenge the cutoff all give the correct response. An internal method comparison versus samples analyzed via GC-MS (50 positive samples and 10 negative samples n=10) demonstrated 100% correlation. GC-MS confirmed negative clinical samples give an average reading of 39.7 ng/mL.

Conclusion: The above studies demonstrate that this assay provides an accurate and precise method for screening urine for the detection of amphetamines and related compounds on automated systems.

*These assays are currently in development and have not been approved for use in the US by the FDA.

Keywords: amphetamine, methamphetamine, immunoassay

P15. Reversing adsorptive losses of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid in spiked and collected urine samples

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Aims: To study the factors altering the adsorption of carboxy tetrahydrocannabinol (THC-COOH) spiked into urine stored in polypropylene containers used for collection, and to observe whether these losses also occurred after the collection of authentic urine samples from THC users.

Methods: Blank urine samples were adjusted to the various pH values and accurate volumes pipetted into polypropylene urine containers. These were then spiked at various concentrations with a solution of THC-COOH, mixed and stored under various conditions. Analysis of THC-COOH was performed on an Agilent 5973 GCMS after extraction and methylation with iodomethane. Samples were analysed before and after the addition of sodium hydroxide. Fifty collected urine specimens, which had previously been analysed for THC-COOH were reassayed after the addition of sodium hydroxide to the original urine sample in its container.

Results: Factors such as pH, temperature, time of storage and volume of urine had a significant impact on the loss of THC-COOH in spiked urine samples.

At low pH values the loss of THC-COOH was greater than at high pH's (59% at pH 5.0 compared to 3.6% at a pH of 8.0). Low volumes of urine added to the containers also contributed to increased losses of THC-COOH. Urine samples at pH of 5.5 spiked at 50 µg/L and stored at room temperature overnight showed losses ranging from 12% for a volume of 40 mL to 26% for a volume of 10 mL. The temperature used for storage also produced further adsorptive losses such that storage at 4°C resulted in an additional 15% loss compared to storage at room temperature for urine samples with the same pH and volume. The addition of a pellet of sodium hydroxide to each sample completely reversed the adsorptive losses. Overall results from collected urine specimens showed a mean increase of 10% (range 3-41), following the addition of sodium hydroxide.

Conclusions: Significant losses of up to 59% occurred when blank urine was spiked with THC-COOH and stored in the polypropylene urine collection containers used in our laboratory service. These losses were dependent on urine pH, volume and storage conditions. Low pH, low volume and storage for 12 hours at 4°C resulted in the highest loss. The percentage loss was similar regardless of the THC-COOH concentration. Understanding adsorptive losses can be beneficial when preparing spiked calibrators and controls where sampling occurs from the primary container. Losses could be readily reversed by the addition of sodium hydroxide. In urine samples from THC users, adsorptive losses of unconjugated THC-COOH were significantly lower (mean 10%) than seen with spiked samples, presumably because authentic urine samples have only low concentrations of this analyte.

Keywords: carboxy tetrahydrocannabinol, adsorption, urine

P16. Comparison of extraction recovery and LC-MS matrix effects of 20 antipsychotics using liquid –liquid and solid-phase extraction methods

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Introduction: Antipsychotic drugs are frequently associated with sudden death investigations. Detection of these drugs is necessary to establish their use and possible contribution to the death. LC-MS(MS) methods are common, however accurate and precise quantification is assured by using validated methods. Validation studies have usually been performed in ante-mortem blood. The aim of this research was to compare extraction recovery and matrix effects using common liquid-liquid and solid phase extraction procedures in both ante-mortem and post-mortem specimen using LC-MS/MS.

Methods: Ante-mortem (AM), non-decomposed post-mortem (PM), and heavily decomposed post-mortem (DC) blank blood specimens were used. Extraction efficiencies and matrix effects were determined in five different blank blood specimens of each blood type. The samples were extracted using a number of different liquid-liquid extraction methods and compared to a standard mixed-mode solid phase extraction method. For liquid-liquid extraction, 500 µL blood was diluted with 1 mL Trizma buffer (pH 9), 1 mL saturated Na_2SO_4, or $NaHCO_3$ and extracted with n-butylchloride, ethylacetate, or ethylacetate:diethylether (1:1). For solid phase extraction, the drugs were extracted using High Flow Slean Screen® solid-phase extraction columns from UCT. The extracts were separated on a Hypersil® C18 column and gradient elution using an Agilent 1200 HPLC. The analytes were detected using an Applied Biosystems 3200 Q-Trap mass spectrometer operated in MRM mode recording three MRM transitions per analyte. Matrix effects were determined using a post-extraction addition approach, the blank blood specimens were extracted as described above and the extracts were reconstituted in mobile phase containing a known amount of analyte.

Results: For most analytes, the extraction recovery using the different liquid-liquid extraction procedures, Trizma buffer and butylchloride yielded the best results. Also in terms of matrix effects, this extraction gave the best recovery for most analytes. The extraction with ethyl acetate showed low extraction recoveries and significant matrix effects (especially ion suppression). Solid phase extraction compared favourably to the best liquid-liquid extraction method in terms of extraction recovery and matrix effects. The extraction comparison of ante-mortem and post-mortem blood showed significant differences, in particular the extraction recovery was quite different between ante-mortem and post-mortem blood. Aripiprazole for example showed extraction recoveries of approx. 55% after liquid liquid extraction in AM and PM blood, whereas the recoveries were only 15% in some DC blood samples after liquid-liquid extraction. In general, matrix effects did not vary as much as the extraction recoveries, except one DC blood sample which showed very significant ion suppression after liquid-liquid extraction. In this sample, only 0.1% of the signal of post-extraction added olanzapine could be detected. Sulpiride showed generally extraction recoveries of less than 10% using liquid-liquid extraction, whereas using solid-phase extraction, the recoveries were approx. 50%.

Conclusion: Quantitative methods used for determination of antipsychotic drugs in post-mortem blood should establish that there are no differences in extraction recovery and matrix effects, particularly if using ante-mortem blood as calibrant.

P17. Analysis of drugs and poisons by LC-TOF-MS: preliminary studies on mushroom toxin alpha-amanitin

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Introduction: Alpha-amanitin is a mushroom toxin with a cyclic peptide consisting of eight amino acids (MW=919). It is possibly the most deadly of all amatoxins found in several members of the Amanita genus of mushrooms.

Methods: The authentic alpha-amanitin solution at 1 mg/mL was prepared in methanol and stored in the dark at -20°C. The working standard solutions were prepared at 1 mcg/mL, 100 ng/mL, 10 ng/mL from the stock solution. The solutions were freshly prepared for each analysis. A Qstar XL hybrid LC-MS/MS system was used for analysis. This system enabled both an LC-single TOF-MS mode and an LC-tandem Q-TOF-MS. The separation column used was L-column ODS (2.1 x 150 mm, particle size 5 micrometer). The following gradient system was used with a mobile phase A (10 mM ammonium acetate (pH 5.0)) and mobile phase B (acetonitrile) delivered at

0.2 mL/min; A:B=100:0 (0 min) – 70:30 (8 min). The injection volume was 5 µL. MS analysis was used in the positive mode.

Results: Under these LC conditions, alpha-amanitin appeared at the retention time of 12.7 min. In the LC-single TOF-MS mode, the mass spectrum of alpha-amanitin showed the molecular base peak at m/z 919.3787 and a relatively intense adduction at m/z 936.4052. Therefore, preliminary experiments were performed for mass chromatographic detection of alpha-amanitin in the standard solutions at low concentrations using the mass slit at m/z 919.135 to 919.635. In the mass chromatogram, the peak shape was symmetrical, and the detection limit of alpha-amanitin was found as low as 0.05 ng in an injected volume. The selection of an internal standard, detailed procedure for extraction of alpha-amanitin from biological samples, calibration curves and validation of this method are now under investigation in our laboratory.

Conclusion: Alpha-amanitin is a cyclic peptide toxin with a relatively high molecular weight. The present LC-TOF-MS seems very suitable for highly sensitive detection of peptide toxins with molecular weight around 1,000 daltons. This method will be a powerful tool for detection and identification of amatoxins, because of the suitability for compounds with high molecular weights, high resolution and high sensitivity of a new TOF-MS instrument.

Keywords: TOF-MS, amanitin, mushroom

P18. HPLC-MS and 1H NMR analyses in a case of carbofuran and metaldehyde poisoning

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Aims: Carbofuran is a broad-spectrum carbamate insecticide that inhibits cholinesterase enzymes and so, can be very toxic to humans. Metaldehyde is a molluscicide bait less toxic to humans than carbofuran. The aim of this work is to check the applicability of HPLC-MS and ^{1}H NMR spectroscopy to detect and quantitate these compounds in suspected ingested granules and in a biological sample. These investigations were developed in order to clarify the origin of an acute poisoning. A 54 year-old woman was found dead at home. A bottle of alcohol and two types of granules, green and blue, were found near her body. Only blood sample, drawn after death, was available.

Methods: HPLC-MS analyses were performed on a XTerra MS C_{18} column using Waters Alliance LC-MS system. Extraction was done in basic buffer with methylclonazepam as internal standard. NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at ambient probe temperature. Green and blue granules were partly dissolved into D_2O. Blood sample was deproteinised by sulfosalicylic acid directly added and NMR analyses were performed on the supernatant. Quantitations could be performed relatively to a 3-trimethylsilyl 2,2,3,3-tetradeuteropropionic acid titrated solution in D_2O.

Results: For standard carbofuran in solution, main resonances were two singlets, one at 1.5 ppm assigned to $(CH_3)_2$ and the second at 2.8 ppm (CH_3-NH). Aromatic protons of the molecule gave resonances between 6.9 and 7.2 ppm. Metaldehyde was characterized by a doublet at 1.35 ppm assigned to the methyl protons, and a quadruplet at 5.29 ppm assigned to the methine group. The two types of granules were analysed by ^{1}H NMR spectroscopy: green granules revealed the presence of carbofuran whereas in blue ones, metaldehyde was found. Results by HPLC-MS confirmed the ^{1}H NMR spectroscopy results. Analysis of cadaveric blood by HPLC-MS revealed, besides ethanol at 2.15 g/L, the presence of carbofuran (retention time: 7.9 min) estimated to 1.4 mg/L as well as small amounts of it's major metabolite: 3-hydroxy carbofuran (RT: 4.2 min). Moreover, no signal corresponding to metaldehyde could be observed. By ^{1}H NMR spectroscopy, presence of ethanol was confirmed; no signal corresponding to carbofuran or metaldehyde was detected, but other unusual signals were observed: a doublet at 2.25 ppm and a quadruplet at 9.68 ppm. These signals were assigned, respectively, to methyl protons and aldehydic proton of acetaldehyde (ethanal). Relative integration of CH_3 protons gave an acetaldehyde concentration of 0.130 mmol/L (normal value below 1 µmol/L). As the blue granules found near the body contained metaldehyde, this compound was probably ingested and readily converted to acetaldehyde in the circulatory blood.

Conclusions: HPLC-MS is an efficient tool to detect carbofuran in cases of acute poisoning as this technique is much more sensitive than ^{1}H NMR spectroscopy. Metaldehyde was not detected at that time. Acetaldehyde, detected by ^{1}H NMR spectroscopy showed that metaldehyde was probably also ingested. The presence of huge amounts of acetaldehyde was observed by ^{1}H NMR in a previous fatal case (unpublished work). The present work illustrates the complementarity between the two techniques in the diagnosis of acute poisonings and more generally in forensic toxicology.

Keywords: carbofuran, metaldehyde, blood

P19. Single-step procedure for the determination of 18 amphetamine-type stimulants and related drugs in body fluids: an update with fast multidimensional gas chromatography-mass spectrometry

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Introduction: Previously, we have described a rapid single-step pre-treatment procedure for assay of wide variety of amphetamine-type stimulants (ATSs) and structurally related common medicaments in body fluids by gas chromatography-mass spectrometry (GC-MS). However, while improvements in the efficiency of pre-treatment allow larger numbers of samples to be prepared in a day, the chromatographic analysis may become a bottleneck. The aim of this study was to speed-up the separation method using multidimensional GC, while maintaining sufficient chromatographic resolution. The performance of the instrumental configuration and analytical method was evaluated in validation experiments, as well as analyses of authentic samples.

Methods: The sample pre-treatment was performed as described earlier. Briefly, the drugs were extracted from their matrices and derivatized in a single step using the following procedure: a 200 µL aliquot of sample was mixed with 50 µL of alkaline buffer and 500 µL of extraction-derivatization reagent (toluene + heptafluorobutyric anhydride + internal standard), centrifuged, and injected into a GC-MS apparatus. The GC was equipped with a Deans switch device, an inert mass selective detector with electron impact (EI) ionization chamber and an autosampler. The chromatographic separations were performed using a 5-m DB-35MS (0.32 mm i.d., 0.25 µm film thickness) column as a pre-fractionating primary column and a 15-m DB-5MS (0.25 mm i.d., 1 µm film thickness) secondary analysis column. A 3.2-m deactivated empty capillary column (restrictor) of 0.18 mm i.d. connected into the exit gas line was used to create optimum pressure conditions. The column temperature was initially 130°C with a hold time of 1 min, and was increased 45°C/min to 310°C. The total run time was 5 min. The Deans switch valve was switched on at 0.40 min to move the analytes to the secondary column, and switched off (to direct the flow to the restrictor) at 3.5 min.

Results: The ATSs and related drugs were identified by their retention times, target and qualifier ions, and their ion ratios. Neither selectivity problems nor matrix interference were observed although the run time was reduced to 5 min. This is less than one third of the chromatographic run time of our previously presented GC-MS method for the same group of analytes. The validation experiments showed that both accuracy and precision of the method are acceptable, i.e. both bias and relative standard deviation (RSD) were below 15% (below 20% at the 25- or 50 ng/mL LLOQ) for all the analytes.

Conclusion: This study shows that it is possible to speed-up the chromatographic method with fast multidimensional GC without compromising the reliability of analytical method performance. After more than 15,000 successful ATS analyses since its introduction in September 2006, this method has been proven reliable enough to be used in large scale in a routine toxicological laboratory. The solvent front and high-boiling components were not channelled into the secondary analysis column, which increases column life, reduces the need for MS ion source cleaning, and decreases the bakeout time.

Keywords: amphetamines, multidimensional gas chromatography, mass spectrometry

P20. ToxID 2.0 Software for the identification of analytes in various LC-MS based general unknown screening applications

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Introduction: Many clinical and forensic laboratories utilize general unknown screening (GUS) to identify analytes present in biological samples. The emergence of LC-MS in these labs has led to the implementation of various mass spectrometer based detector strategies for GUS. ToxID 2.0 software was designed to automate various LC-MS based GUS acquisition methods and allow for fast, error-free data analysis and report generation.

Methods: ToxID 2.0 can support GUS data processing and report generation on ion trap, triple quadrupole and Orbitrap mass spectrometers. With ion trap mass spectrometers, two screening methods are supported. One method utilizes full scan MS2 spectra and the other uses both MS2 and MS3 spectra for searching against a known spectral library of compounds. With the triple quadrupole mass spectrometer, two GUS methods are also supported. In the first method a user defined SRM intensity threshold activates full scan MS2 scans, which are used to search against a library. The second triple quadrupole method uses the three most intense H-SRM (highly-selective reaction monitoring) transitions as well as ion ratios to identify each analyte. With the Orbitrap mass spectrometer, full scan accurate mass data is used to identify each analyte. ToxID 2.0 software combines mass spectrometer data and LC retention times to positively identify samples and produce short summary and long detailed reports.

Results: For all instrument acquisition methods described in Methods, the screening of 300 compounds was validated by processing and analyzing urine samples spiked with 10 randomly selected compounds at various concentrations. Each method was also confirmed with real patient samples. ToxID 2.0 software was used for data analysis and report generation in all the supported GUS methods. With ToxID 2.0, data analysis is fast and error-free. Table 1 below shows a sample set of data obtained on a LXQ ion trap, with MS2 based identification.

Table 1. Sample set of data collected on LXQ with MS2 based ID.

Compound	10 ng/mL in Urine	100 ng/mL in Urine	1000 ng/mL in Urine
Cocaine	P	P	P
Nicotine	P	P	P
Nicardipine	P	P	P
Norcodeine	P	P	P
OH-LSD	N	P	P

*N=drug not present P=drug present

Conclusion: ToxID 2.0 is a versatile software for use in data processing and report generation in a variety of LC-MS based general unknown screening methods in toxicology and forensic laboratories.

Keywords: general unknown screening, toxicology, LC-MS

P21. Workup of urine samples for systematic toxicological analysis using GC-MS: acid hydrolysis/liquid-liquid extraction/acetylation versus acid hydrolysis/solid-phase extraction/acetylation, enyzmatic hydrolysis/solid-phase extraction/acetylation, and enyzmatic hydrolysis/extractive methylation

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Introduction: Sample preparation for Systematic Toxicological Screening Analysis in urine (U) by GC-MS generally involves cleavage of conjugates by acid hydrolysis (Hy) or enzymatic hydrolysis (Gluc) followed by liquid-liquid extraction (LLE) or solid-phase extraction (SPE), and derivatization, e.g. acetylation (Ac). LLE and derivatization can be performed simultaneously, e.g. extractive methylation (ExMe). The aim of the presented work was a systematic comparison of the standard workup in our laboratory (U-Hy-LLE-Ac) and 3 alternative workup procedures (U-Gluc-SPE-Ac, U-Hy-SPE-Ac, and U-Gluc-ExMe).

Methods: The study consisted of two parts. The 350 urine samples used in the first part were obtained from 168 in-patients from an internal medicine ward. One part of the samples (5 mL) was worked up by U-Hy-LLE-Ac using 10 *M* HCl for hydrolysis (15 min) and extraction with ethyl acetate-dichloromethane-isopropanol (3:3:1, v/v/v) at pH 8-9. The extract was evaporated and acetylated using acetic anhydride-pyridine (3:2, v/v) and microwave irradiation. Another part (1 mL) was worked up by U-Gluc-SPE-Ac using glucuronidase/arylsufatase (50°C, 90 min) for Gluc and Isolute Confirm HCX cartridges (130 mg, 3 mL) for mixed-mode SPE. The acidic/neutral fraction eluted with methanol and the basic fraction eluted with methanol-aqueous ammonia (98:2, v/v) were combined, taken to dryness, and acetylated as described. A third part (2 mL) was worked up by U-Gluc-ExMe at pH 12 with 6 mL of 1 *M* methyl iodide and tetrahexyl ammonium hydroxide as phase transfer catalyst (PTC). In a follow-up study, 100 urine samples from psychiatric in-patients were used after routine analysis had been completed. One part (5 mL) was worked up by U-Hy-LLE-Ac as described. Another part (2 mL) was worked up by U-Uhy-SPE-Ac, a combination of acid hydrolysis and mixed-mode SPE as described above with slight modifications. After reconstitution in methanol, all samples were analyzed by full-scan GC-MS (HP-1 column, 12 m × 0.2 mm I.D; scan range *m/z* 50-550). The detected drugs and/or their metabolites/artifacts were listed in MS Excel tables and compared.

Results: Between U-Hy-LLE-Ac and U-Gluc-SPE-Ac, there were only very few differences in the spectrum of analytes detected apart from salicylic acid being the only drug not detectable with the latter. U-Hy-SPE-Ac also covered a wide spectrum of analytes but several compounds detectable with U-Hy-LLE-Ac were not detectable after this workup, e.g. paracetamol. As expected comparison of U-Hy-LLE-Ac and U-Gluc-ExMe showed that the earlier was better suited for basic drugs and the latter for acidic drugs, but the overlap was considerable. Workup by the SPE procedures as well as U-Gluc-ExMe was time-consuming requiring 1 hour or longer to perform, while U-Hy-LLE-Ac could be completed in about 35 min. The 2 SPE methods yielded cleaner chromatograms, facilitating data evaluation.

Conclusion: U-Hy-LLE-Ac remains the method of choice for STA in clinical toxicology due to its wide analyte spectrum and short workup time. U-Gluc-ExMe is an ideal complimentary method when acidics need to be covered also. U-Gluc-SPE-Ac can be used as an alternative to U-Hy-LLE-Ac when turnaround is not critical or when automated analysis is required.

Keywords: GC-MS, urine, extraction, workup

P22. Accurate determination of opiates by direct injection of urine using UPLC-MS/MS

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Introduction: Detection of opiate intake is a major objective of urine drug testing. Gas chromatography-mass spectrometry with hydrolysis of glucuronides is used for confirmation of positive results from screening analysis of morphine, morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G), codeine, codeine-6-glucuronide (C6G), 6-monoacetylmorphine (6MAM), ethylmorphine and ethylmorphine-6-glucuronide (EtM6G). Liquid chromatography-mass spectrometry could be an alternative method performed with simplified sample preparation procedures.

Methods: A direct ultra-performance liquid chromatographic-tandem mass spectrometric method (UPLC™-MS/MS) for measurement of urinary opiates was developed. The LC-MS/MS system consisted of a Waters Acquity Ultra-performance liquid chromatograph connected to a Quattro Premier XE tandem mass spectrometer (Waters Co). Chromatography was performed using a 1.8-µm 100 x 2.1 mm (inner diameter) HSS T3 C18 column (Waters Co), preceded by 0.2 µm column filter (Waters Co). The sample preparation consisted of mixing 25 µL urine and 100 µL of deuterium-labelled internal standards. Instrumental features were reversed phase chromatography with gradient elution, electrospray ionisation, and monitoring of two product ions in selected reaction monitoring (positive) mode. A comparison with the reference GC-MS method was done using 200 authentic patient urine samples, which were positive in the immunochemical screening assay for opiates.

Results: The measuring range was 50 – 50 000 ng/mL for morphine, M3G, M6G, codeine, C6G, ethylmorphine, EtM6G and 2 – 2500 ng/mL for 6MAM. The intra- and inter-assay imprecision, expressed as the coefficient of variation was below 6.5% for all compounds (N=15). Influence from urine matrix (up to 10% reduction of signal for the first eluted compound M3G) was noticed in infusion experiment, but was compensated for by the use of the co-eluted internal standards. Comparison with the GC-MS method (within measuring range 150 – 5000 ng/mL for morphine, codeine, ethylmorphine and 10- 200 for 6MAM) in 200 authentic patient samples confirmed the accuracy of the method with a mean ratio between methods (GC-MS to UPLC-MS/MS) of: 0.99 (r^2=0.966, N=130) for total morphine; 1.03 (r^2=0.973, N=52) for total codeine; 1.12 (r^2=0.958, N=11) for total ethylmorphine; 0.99 (r^2=0.991, N=10) for 6MAM. No false positive or negative results were obtained with the new method. A number of 20 authentic patient urine samples, being negative in the immunochemical screening assay, confirmed not to contain any detectable opiate compound. No interference for morphine, M3G, M6G, codeine, C6G, ethylmorphine, EtM6G and 6MAM was observed analyzing the spiked sample containing 4000 ng/mL of the following substances: hydrocodone, hydromorphone, dihydrocodeine and oxycodone.

Conclusion: A fully validated and robust method for direct measurement of opiates in urine was developed. The UPLC-MS/MS method enabled accurate identification and quantification of opiates, including 6MAM, in urine.

Keywords: urine, GC-MS, UPLC-MS/MS

P23. Evaluation of Bio-Rad TOX/SeeTM rapid urine drug screen for screening tricyclic antidepressants in emergency toxicology

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Introduction: Intoxication by Tricyclic Antidepressants (TCA) is a frequent diagnosis in emergency rooms worldwide. Liquid chromatography/Mass Spectrometry (LC/MS) and high performance liquid chromatography (HPLC) can provide specific identification of TCA. However, both LC/MS and HPLC are used infrequently in emergency rooms to screen TCA intoxication due to consideration of turn around time, instrumentation cost, and on-site accessibility. Therefore, there is a need for a simple, rapid and portable screening device that can detect a broad range of commonly prescribed TCA. We evaluated Bio-Rad TOX/See Rapid Urine Drug Screen device for analyzing seven common TCA in patient urine, including amitripyline, nortriptyline, desipramine, imipramine, doxepin, clomipramine and opipramol. opipramol is primarily prescribed in Germany.

Methods: TOX/See is a single-step, lateral flow chromatographic immunoassay for the qualitative detection of drugs and metabolites in urine. It provides test results in 5 minutes with a detection cutoff of TCA at 1000 ng/mL. The purpose of this study was to investigate the broad screening range of TOX/See TCA assay. We evaluated 63 urine samples with known HPLC result of TCA by TOX/See. Among these 63 urine samples, 20 were obtained from a reference lab in the USA, and 43 were from Germany. All 63 urine samples were analyzed by an HPLC system, REMEDi-HS™, to determine the specific TCA and concentration.

Results: TOX/See gave expected results for Bio-Rad Liquichek™ Qualitative Urine Toxicology Controls. Thirty-four out of 63 samples were positive for TCA by TOX/See, including seven commonly prescribed TCA identified by HPLC: amitripyline, nortriptyline, desipramine, imipramine, doxepin, clomipramine and opipramol. The detection of opipramol in urine by TOX/See TCA assay was not reported previously. Among 29 TOX/See negative samples, 21 had no detectable amount of TCA by HPLC, 8 had TCA concentrations below 1000 ng/mL (ranging from 68 to 801 ng/mL) for amitriptyline, nortriptyline, despiramine and doxepin metabolites by HPLC.

Conclusion: The agreement of TOX/See with HPLC demonstrated the usefulness of TOX/See in screening a broad range of TCA at emergency room. The self-contained TOX/See cassette eliminates the downtime of instrumentation, and TOX/See's shorter assay time streamLines testing.

Key Words: tricyclic antidepressants, emergency toxicology, screening

P24. Determination of GHB in urine by HS-GC-FID

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Aim: To develop a HS-GC-FID method for determination of exogenous intake of gamma-hydroxybutyrate (GHB) in forensic urine samples.

Methods: A headspace gas chromatography flameionisation (HS-GC-FID) method was developed for determination of GHB in urine. Conversion of GHB to gamma-valerolactone (GBL) was carried out before liquid-liquid extraction (LLE). An aliquot of 100 µL urine was added 60 µL of concentrated H_2SO_4. The sample was then extracted with 400 µL dichlormethane:hexane solution (80:20) and 100 µL of the organic layer was transferred to a 20-mL headspace vial. The samples were heated in the autosampler (Turbomatrix, PerkinElmer) for 10 minutes at 100ºC prior to injection. A GC-FID system (Clarus 500, PerkinElmer) with a Rtx-BAC1 column (30 m, 0.32 mm i.d, 1.8 µm d.f., Restek Corp.) and a temperature gradient from 90 to 150ºC was used to separate GHB and the internal standard α-methylene-γ-butyrolactone within 6 minutes.

Results: The calibration curve of extracted urine standards was linear for the concentration range 10 to 400 mg/L. The within-assay precisions for the working range were 1.0 to 1.9% RSD. Between-assay precisions and accuracies obtained during a two month period with one replicate on 10 different assays, were 2.9 to 5.2% RSD and 94 to 106% for the whole working range, respectively. The robustness of the LLE and HS methods were found satisfactory using full factorial designs. Method comparison using a different GC-FID method utilizing liquid injection was carried out. This comparison showed satisfactory agreement when analyzing GHB positive authentic forensic urine specimens obtained from living and dead people.

Conclusion: This rapid, robust and reproducible method proved to be suitable for the determination of GHB in forensic urine cases. The method is

used for routine forensic analysis for investigations of suspicion of drug rape and/or intoxication.

Keywords: GHB, HS-GC-FID, urine

P25. An on-line turbulent flow extraction LC-MS/MS screen for the quantitative analysis of multiple classes of illicit drugs in both plasma and urine

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Introduction: Drugs of abuse in human biological fluids are most commonly quantified by GC-MS. GC-MS analyses, however, require extensive sample cleanup and a lengthy derivatisation. Recently, forensic and toxicology laboratories have moved to LC-MS analyses to eliminate the derivatisation step, but a rigorous solid-phase extraction cleanup step is still required. In addition, many LC-MS/MS methods for the analysis of drugs of abuse only assay one class of drug compound. On-line turbulent flow technology allows the direct injection of biological matrices onto the LC-MS system, and thus, sample preparation is minimal. Here, we describe an on-line turbulent flow extraction LC-MS/MS method that may be used to quantitatively analyse over twenty compounds from many classes of drugs of abuse (including opiates, stimulants, depressants and some of their primary metabolites) from plasma and urine.

Methods: A mixture of illicit drug compounds (including amphetamines, benzodiazepines, cocaine and opiates) were spiked into both plasma and urine matrices. Turbulent flow extraction was performed on an Aria TurboFlow™ TLX 1 system (Thermo Fisher Scientific). 10 µL of raw sample was injected onto a Cyclone-MAX 0.5 x 50 mm extraction column (Thermo Fisher Scientific) before being back-flushed onto a Hypersil™ GOLD aQ 2.1 x 50 mm, 5 µm analytical column (Thermo Fisher Scientific). Turboflow LC conditions were as follows: eluent A - 0.1% formic acid, eluent B - 10 mM ammonium bicarbonate (pH9) and eluent C - 0.1% formic acid in acetonitrile with a flow rate of 1.5 mL.min^{-1} during loading. Analytical LC conditions were as follows: eluent A - 0.1% formic acid and eluent B - 0.1% formic acid in acetonitrile with a flow rate of 300 uL min^{-1}. The entire LC effluent from the sample injections was directed to the Ion Max™ source, utilising heated electrospray ionisation on a TSQ Quantum Ultra™ triple quadrupole mass spectrometer in positive ion SRM mode.

Results: A panel of drugs of abuse from plasma and urine samples were detected at 1 - 10 ng/L concentration levels in a single LC-MS/MS run.

Conclusion: A fast (data acquisition time <10 minutes) and sensitive method for the detection of multiple classes of illicit drugs and their metabolites in plasma and urine is described. Minimal sample preparation is required and the assay covers a wide concentration range (1 – 500 ng/mL).

Keywords: Drugs of abuse, on-line extraction, LC-MS/MS

P26. Carbon monoxide concentrations in post mortem tissues

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Introduction: When blood samples are not available, measurements of carbon monoxide (CO) concentration tissues can be used as markers for the degree of exogenous CO exposure and the identification of possible causes of death. The aim of this work is to present our experience in consideration of the interpretation difficulties and possible bias.

Methods: CO determination assays in tissues were performed using a headspace (HS) technique coupled with- gas chromatography - mass spectrometry (GC/MS) as follow: 2 g of diced tissue (2 mm length) were put in a 22 mL HS-vial, and after H_2SO_4 addition, CO was introduced in the chromatographic system using an automated HSTurbomatrix40 headspace sampling system (Perkin-Elmer, Norwalk, CT, U.S.A.). Separation was performed on a 30 m x 0.32 mm i.d. RT-Msieve 5A column (Restek, France) and detection used a QP2010 detector (Shimadzu, Japan) in the SIM mode (m/z 28). CO quantitation was performed using an external formic acid calibration. In our unit, we did a retrospective examination of forensic cases including CO determinations in various post mortem tissues. In addition, we performed a limited study to investigate CO stability in post mortem tissues at 3 temperatures (room temperature, 4°C and -20°C) for one month.

Results: The forensic cases were classified according to the 4 categories: death not due to a fire without CO intoxication; death in a fire, without CO intoxication; death with CO intoxication, but not in a fire; death with CO intoxication, in a fire. The results are presented in the table (CO concentration in µL/g of tissue; nv: no value; sv: one value):

	Lungs	Muscle	Liver	Brain	Heart	Kidney
Death not due to a fire, without CO intoxication (n=6)						
mean	4.8	2.0 (sv)	0.9 (sv)	12.7 (sv)	0.002 (sv)	nv
extreme	2.5 – 6.1					
Death in a fire, without CO intoxication (n=3)						
mean	2.7	1.3 (sv)	nv	nv	0.02 (sv)	nv
extreme	0.002 – 6.6					
Death with CO intoxication, not in a fire (n=3)						
mean	60.5	3.8	nv	nv	nv	3 (sv)
extreme	49-72	2 – 6.5				
Death with CO intoxication, in a fire (n=3)						
mean	39.2	nv	18	2.6 (sv)	7.6 (sv)	nv
extreme	24.4-61		11-25			

The preliminary results of our restricted study of stability show a global increase tendency of the CO concentrations in tissues. However, CO concentrations seem to be relatively stable when initial CO concentrations are high (i.e. in lungs of CO intoxication cases).

Conclusion: According to other authors, lungs appear as a helpful tissue to determine CO exposition, certainly owing to their high blood irrigation. Regarding stability, we know that CO can be produced in post mortem tissues by putrefaction and/or be lost at room temperature. Owing to the results of our limited study, this instability risk remains difficult to evaluate. In addition, there is probably a bias due to the selection of organ samples. According to these elements and to the limited data in the literature, special care should be taken when interpreting the results.

Keywords: carbon monoxide, post mortem, tissues

P27. Carbon monoxide-related death in Southern Spain: a 7-year study

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Introduction: Carbon monoxide (CO) is a colorless, odorless, tasteless toxic gas produced by incomplete combustion in fuel-burning devices such as motor vehicles, gas-powered furnaces, and portable generators. Exposure of humans to high concentrations of carbon monoxide can result in death, due to the formation of carboxyhemoglobin (COHb), which impairs the oxygen carrying capacity of the haemoglobin. Carbon monoxide is responsible of a great number of accidental domestic poisonings and deaths throughout the world, particularly in homes that have faulty or poorly vented combustion appliances. The objective of this study was to determine the incidence of carbon monoxide-related deaths in forensic cases received in the Department of Seville of the Spanish National Institute of Toxicology from 2000 to 2006. During the period studied, a total of 21672 cases received in our laboratory for toxicological analysis, in 515 (2.38%) of them carbon monoxide determination were requested. These forensic cases included: traffic accidents, accidental poisoning, suicide cases and fire deaths.

Methods: Due to the fact, that only a small amount of absorbed CO is present in blood in a free state, its determination is normally done by analysing carboxyhemoglobin (COHb). Carboxyhemoglobine levels were determined in blood using the normal procedure in our laboratory, which consists in a fourth derivative spectrophotometric method, using sodium ditionite as releasing agent. COHb levels were then measured after the saturation of blood with carbon monoxide.

Results: In 415 of these cases (81%) carboxyhemoglobin was detected. During the period studied, we observed that the numbers of cases has been continuously increasing from a 6.5% in 2000 to a 22% in 2005. However, a decrease in the incidence has been observed in 2006 with a 10% of the cases. Carboxyhemoglobin (COHb) levels obtained were divided in two groups. One included cases where COHb levels were above 50% and the other one cases which COHb levels below 50%. COHb levels above 50% are considered high enough to explain the death cause. In a 33% of the cases COHb was above 50% and in a 48% of the cases was below 50%. During the period studied, males represented the big majority 72% of deceased people and in a 35% of them, COHb levels were above a 50%. In a 26% of the cases the deceased was a female.A 26% of them resulted in a COHb levels above 50%. Sex was unknown in 2% of the cases. Death related to carbon monoxide intoxication were prevalent (16% of the cases) in the young people (0-20 years) presenting a 30% of these cases, levels of COHb above a 50% and also in elderly (>60) (27.5% of the cases) with a 16% of the cases which resulted in a COHb level above 50%.

Conclusions: Our results indicate that improved population-based prevention measures, including educating the public about the dangers of CO exposure, are needed in order to avoid domestic accidental deaths.

Keywords: carbon monoxide, death, Spain

P28. Postmortem distribution of meprobamate

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Introduction: Meprobamate is a mild-sedative-hypnotic drug used in therapy since the 1950s. Being widely known in France and particularly in use in suicide attempts, its identification and quantitation in biological fluids represent a routine operation for the clinical and/or forensic toxicologist. However, although fatal poisoning cases are well described in the literature, a limited amount of scientific information concerning the *postmortem* tissue distribution of meprobamate has been reported to date. Additionally, none of these data pertain to therapeutic levels of the drug. As a preliminary study, our laboratory has determined the distribution of meprobamate in various *postmortem* fluids and tissues collected during autopsy from 8 fatalities that were reported positive for meprobamate in peripheral blood.

Methods: When available, 9 specimen types collected during autopsy were analyzed for each case, including peripheral and cardiac blood, vitreous humor, bile, liver, kidney, lung, heart and brain. All tissue homogenates were prepared by homogenized 200 mg of tissue with 1 mL of saline. Meprobamate was extracted from all samples investigated by a liquid-liquid extraction method using chloroform at pH 12, in presence of carisoprodol, used as internal standard. Analysis of meprobamate was then performed on all fluids and tissues by liquid-chromatography ion-trap tandem mass spectrometry (LC-MS/MS).

Results: Peripheral blood concentrations of meprobamate in these 8 cases ranged from 9 to 160 mg/L. 5 of these cases showed meprobamate peripheral blood concentrations within (n=3) or above (n=2) the known therapeutic concentrations reported in the literature. (i.e. 10-30 mg/L), with no obvious contribution of meprobamate to the cause of the death. In 3 cases, meprobamate was considered to have largely contributed to the cause the death with peripheral blood concentrations above 95 mg/L. The *postmortem* distribution coefficients for meprobamate, expressed as specimen/peripheral blood ratio, are presented below [(mg/L) / (mg/kg)].

	CB*/ PB**	VH***/ PB	Bile/ PB	Liver/ PB	Kidney/ PB	Heart/ PB	Brain/ PB	Lung/ PB
n	8	6	8	6	6	6	6	6
Mean	0.97	0.83	1.16	2.63	1.82	1.81	1.83	1.74
CV (%)	16	20	23	31	19	24	22	20

*CB: cardiac blood - ** PB: peripheral blood – *** VH: vitreous humor – CV: coefficient of variation (%)

Conclusion: With a CB/PB concentration ratio averaged 1, this finding is in agreement with the literature and confirms that meprobamate apparently does not exhibit significant *postmortem* redistribution. The distribution coefficients determined in bile and vitreous humor suggest that meprobamate does not accumulate in those biological fluids. Other tissues concentrations were found higher than peripheral blood concentrations as showed by distribution coefficients for meprobamate ranging from 1.7 to 2.6, and are consistent with the known moderate volume of distribution of this compound (i.e. 0.7 L/kg). Despite our limited number of cases, the relatively small CV values (<25%, except for the liver) associated with these distribution coefficients suggest that these specimens may be used with extreme caution to obtain an approximate peripheral blood meprobamate concentration ranging from therapeutic to toxic levels. Admittedly, a study involving a greater number of samples from a larger pool of cases needs to be completed in order to more definitely verify these results.

Keywords: meprobamate, *postmortem* toxicology, tissue distribution

P29. Fatal case of a 27-year-old male after taking iboga roots in addiction treatment. Determination of ibogaine and ibogamine in biological fluids by GC-MS/MS

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Introduction: Iboga or *Tabernanthe iboga* is a small shrub from the equatorial forests of West Africa. It is the basis of the syncretic Bwiti cult; some tribes in the Congo and Gabon use it in religious rituals to communicate with the spirits of their ancestors. The bark of the dried roots is mixed with other plants and either consumed as it is, or in drinks. The roots of Tabernanthe iboga contain a dozen alkaloids, including ibogaine, the indolic alkaloid that acts as a stimulant in small doses and causes hallucinations in larger doses. Its therapeutic use has been studied for several years in Europe, particularly as a treatment for drug addiction. This report focuses on the death of a 27-year-old drug addict in July 2006 after the consumption of Iboga roots as part of his withdrawal treatment to methadone (La Voulte, France).

Methods: A complete toxicological analysis of the blood and urine was carried out from the autopsy. The analysis of ibogaine and ibogamine was carried out by impact electronic GC-MS/MS after liquid extraction using TOXI-TUBES-A®. Using prazepam-d5 as internal standard, the quantification of ibogaine was done on the ion m/z=225, daughter of m/z=310 and the detection of ibogamine on the ion m/z=195, daughter of m/z=280.

Results: The concentrations of ibogaine found were 1.27 µg/mL in the blood and 1.71 µg/mL in the urine. In addition, toxicological analysis revealed the presence of several central nervous system depressants: diazepam, nordazepam and oxazepam, and methadone, a morphinomimetic, but the combined concentrations were far too weak to cause death.

Conclusion: This is the first GC-MS/MS method for the dosage of these molecules. It has the advantage of increased selectivity and a simple extraction step. The summarization of the histological, anatomopathological and toxicological reports led to the conclusion that ibogaine was implicated in the death, adding to other cases from around the world. After Belgium,

Switzerland and the USA, France now also classes ibogaine as a narcotic (Decree of 12 March 2007, modifying the decree of 22 February 1990 determining the list of substances classified as narcotics).
Keywords: Iboga, death, GC-MS/MS

P30. Deaths related to cocaine consumption: Southern Spanish Experience

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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 a major cause of morbidity and mortality, being cocaine consumption one of the issues that arises higher concern in our society. The objective of this study was to get an insight into the presence of cocaine and related compounds in forensic cases received in the Department of Seville of the Spanish National Institute of Toxicology a year-period. During this year, from a total of 4683, 229 (4.89%) cases, in which drug analysis was requested, gave positive results to cocaine or its metabolites, being the most frequent cocaine consumer is a man (n=180, 78.60%) between 21 and 50 years. In 48.91% of the cases ethanol was also detected.

Methods: Toxicological analyses were performed in all the samples, 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®, then SPE (Bond-Elut, certified) was performed in all the samples and the extracts were analysed by gas chromatography with NPD, high performance liquid chromatography (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS).

Results: 197 (86.02%) cases were cocaine-related deaths Cocaine and its metabolites were detected in 76 of the blood samples, while the metabolites (methylecgonine, benzoylecgonine and ethylbenzoylecgonine) were only detected in 119 (61.57%) of the cases. Ethanol and ethylbenzoylecgonine were detected in 12 cases. Cocaine blood concentrations ranged from 0.02-7.24 mg/L (mean concentration 0.38 mg/L and median concentration 0.12 mg/L). In 41 cases blood cocaine concentration was below 0.1 mg/L, in 25 was between 0.1-0.5 mg/L, in 9 cases between 0.5-1 and in 8 cases over 1 g/L. Benzoylecgonine blood concentrations ranged from 0.01-140.4 mg/L (mean concentration 6'27 mg/L and median concentration 1.25 mg/L).32 (13.97%) of the cases studied corresponded to people deceased in traffic accidents. Cocaine was detected in only 10 of these cases, being benzoylecgonine detected in all of them. Cocaine blood concentrations ranged from 0.01-0.27 mg/L (mean concentration 0.06 mg/L and median concentration 0.03 mg/L). Benzoylecgonine blood concentrations ranged from 0.01-38.74 mg/L (mean concentration 2.07 mg/L and median concentration 0.29mg/L). It is remarkable that in these cases along with cocaine or its metabolites, ethanol was detected in 21 cases. These findings suggest that cocaine consumption was done between 12 and 24 hours before the accident, whereas alcohol was consumed at least 6 hours before it. Ethylbenzoylecgonine was detected in only one of these cases. This fact points out that cocaine and ethanol consumption was not done simultaneously.

Conclusions: As it has been stated before, it seems that with the exception of massive drug exposure, cocaine-related deaths occur for the major part after prolonged drug use and it is almost impossible to correlate a specific blood concentration to toxicity. However, our results some differences between blood cocaine concentrations in people who died in road accidents and those who died in a different manner.

Keywords: cocaine, death due to drug consumption

P31. A fatal poisoning involving Bromo-Dragonfly

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Introduction: A fatal overdose case involving Bromo-Dragonfly is reported. Bromo-Dragonfly is a common name for 1-(8-bromobenzo [1,2-b; 4,5 b´]difuran-4-yl)-2-aminopropane or bromo-benzodifuranyl-isopropylamine. Structurally it is closely related to phenylethylamines like 2C-B and DOB. Bromo-Dragonfly is considered an extremely potent hallucinogen, only slightly less potent than LSD and it has a very long duration of action. The synthesis and its potent agonist character at the serotonin 5-HT_{2A} receptor were described in 1998 [Parker M.A. et al. J Med Chem. 1998; 41: 5148-514]. It is quite a new substance on the drug scene, as the first reports on consumption of this drug appeared in 2005-2006 on more informal internet pages. Recently the drug has also entered the Scandinavian drug scene and two deaths presumably related to intake of Bromo-Dragonfly have appeared in 2007 [Classification document (Dnr: TILLS 2007/63) from Folkhälseinstituttet in Sweden]. In the present case from Denmark an 18-year-old woman was found dead (October 2007). The woman and her boyfriend presumably both ingested 1 mL of a hallucinogen LSD-like liquid the evening before the incident. When the boyfriend woke up at 5 am the following morning he found his girlfriend dead. A medico-legal autopsy was performed on the descendent. Liver, blood and urine were submitted for toxicological examination. Furthermore a bottle containing the hallucinogen LSD-like liquid was subjected for analysis.

Methods and results: The autopsy findings revealed oedema of the lungs, slightly oedema of the brain, enlargement of the spleen, irritation of the mucous membrane in the stomach and ischemic changes in the kidneys. The bottle containing the hallucinogen LSD-like liquid was evaporated to dryness and the residue analysed using UPLC-TOF, GC-MS, HPLC-DAD and ^{1}H-NMR. The analysis indicated the presence of Bromo-Dragonfly. The drug was finally extracted and purified from the liquid. Concomitant GC-MS, HPLC-DAD, 1 and 2D ^{1}H and ^{13}C-NMR analysis and finally elemental analysis confirmed the structure as well as the purity of the isolated compound as Bromo-dragonfly. Liver blood, blood and urine were finally analysed using UPLC-TOF, GC-MS and HPLC-DAD. Bromo-Dragonfly was identified in liver blood and quantified in femoral blood and urine using UPLC-TOF and the isolated compound as reference and calibrator.

Conclusion: Bromo-Dragonfly was isolated and its structure unambiguously determined from a submitted aqueous sample. Subsequently, it was identified in liver blood and quantified in femoral blood and urine in a fatal overdose case. In addition to Bromo-Dragonfly, no ethanol, therapeutic or other drugs of abuse were detected in liver, blood or urine samples from the deceased. The autopsy findings were non-specific for acute poisoning. However, based on the toxicological findings, the cause of death was supposed to be a fatal overdose of Bromo-Dragonfly. To our knowledge, this is the first report of isolation and quantification of Bromo-Dragonfly in biological specimen from at deceased. This case has caused that the drug has been classified as an illegal drug in Denmark since 5th December 2007.

Keywords: Bromo-Dragonfly, bromo-benzodifuranyl-isopropylamine, UPLC-TOF

P32. Heart pathology and fatal drug concentrations. Is there any relationship?

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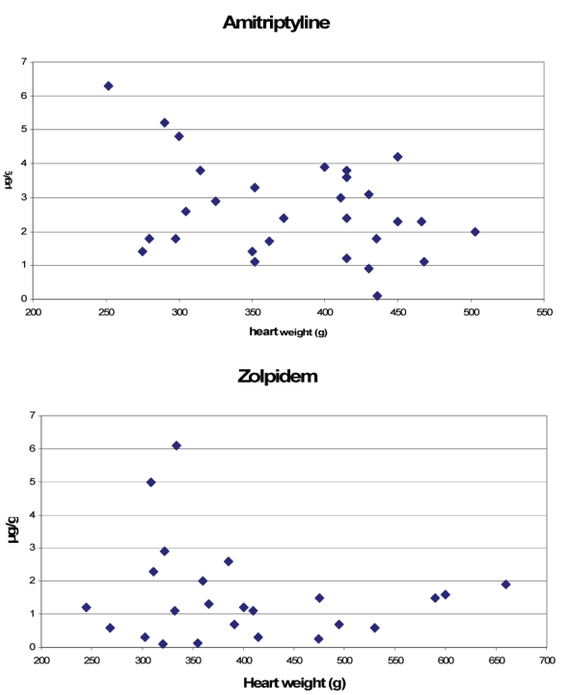
Introduction: It has repetitively been stressed that various pathologies may affect the susceptibility of an individual to the toxic effects of drugs. In order to explain for low concentrations of drugs in obvious or suspected intoxications, pathological conditions are often blamed. Although this

assumption seems logical, there is little scientific evidence to support this view. It is important to realize that if such relationship exist for certain drugs, then the pathophysiological influence must also be logical. Hence, if a drug affecting the heart rhythm, then a heart pathology may be expected to make the subject more prone to contract a severe effect on the cardiac function. Likewise, patients with reduced liver function may be more susceptible to the exposure to drugs targeting the liver. There is, however, no reason to axiomatically assume that such subjects should die at a lower blood concentration of a drug with noxious effects e.g. on the brain. Hence, we decided to study the postmortem concentrations of amitriptyiline and zolpidem, affording toxic effects primarily on the conduction system of the heart, and formatio reticularis of the brain, respectively.

Methods: From the Swedish forensic pathology and forensic toxicology databases, deceased subjects testing positive for amitriptyline or zolpidem by a standard GC method, were selected. Further, cases considered to have died from an intoxication with each of these drugs only, were identified. Decomposed cases were excluded. Since increased heart weight is a generally accepted criterion of heart pathology, the heart weights of these subjects were compiled and compared with the femoral blood concentrations of amitriptyline and zolpidem, respectively. Further, the autopsy protocols were reviewed and the degree of coronary sclerosis was estimated from the descriptions provided by the responsible pathologists.

Results: No correlation between heart weight and femoral blood concentration were found for either drug (see graphs below). Although the highest values for each drugs were observed among subjects with lower heart weights, all other values were randomly distributed across different heart sizes. The blood drug concentrations were not related to the degree of coronary sclerosis either.

Conclusion: No evidence could be obtained to support the notion that heart pathology affects the toxicity of the two drugs investigated. Hence, the assumption that tricyclic antidepressants, such as amitriptyline, with a well-known effect on the QT-interval should be more toxic at lower concentration in individuals with a pre-existing heart condition could not be verified in this study.



Keywords: postmortem toxicology, interpretation, heart disease

P33. L-amphetamine in postmortal blood and urine: a case report

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Introduction: Toxicological interpretation of methamphetamine and amphetamine in postmortal blood may be a challenging task. The presence of methamphetamine is highly indicative of illicit use of "street amphetamine". However, other potential sources exist, as both methamphetamine and amphetamine may be available on prescription for the treatment of disorders such as narcolepsy, attention deficit disorder and obesity. In addition, some drugs may be metabolised to methamphetamine and/or amphetamine. Both methamphetamine and amphetamine are chiral compounds consisting of l- and d-isomers. The l/d-ratio for illicit methamphetamine and amphetamine is usually close to unity, whereas l/d-ratios for prescription drugs are highly variable. Thus, determination of enantiomeric composition may help in interpreting findings. We describe a case where the sole presence of l-amphetamine was helpful in identifying the drug source.

Methods: A 54 year old woman with an unknown previous medical history was found dead at home. Autopsy was performed, and samples of peripheral blood and urine were submitted to our laboratory. Toxicological analyses were performed in blood and urine by LC-MS/GC-MS. The determination of the enantiomeric composition of amphetamine was performed by LC-MS using a chiral column.

Results: The postmortem femoral blood sample contained methamphetamine 0.04 µg/mL and amphetamine 0.02 µg/mL. Urine levels were 1.7 ng/mL and 0.73 ng/mL, respectively. No other drugs were detected. Due to the unexpected presence of sympathomimetic amines in blood and urine, the enantiomeric composition of amphetamine in blood and urine was determined, and revealed the l-isomer only. Further investigation showed that the deceased suffered from Parkinson's disease and used the MAO-B inhibitor deprenyl (selegilin), a known precursor of the l-isoforms of methamphetamine and amphetamine.

Conclusions: The presence of methamphetamine in blood samples is frequently, but not always, associated with illicit drug use. The present case suggests that enantiomer-selective analysis in some instances may yield additional information. This case also illustrates the importance of providing the forensic toxicologist with detailed information on the medical history and drug intake of the deceased.

Keywords: amphetamine, enantiomer, deprenyl

P34. Evaluation of carboxyhemoglobin in ante-mortem and post-mortem burns

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Introduction: Differentiation of ante-mortem from post-mortem burns is very important especially in charred bodies. At the present there is no definite sign for this purpose, although a few criteria can be used as indicators of ante-mortem burns such as presence of soot under vocal cord, carboxyhemoglobin level and vital reaction around burning areas. The main purpose of this study was determination of carboxyhemoglobin which is the most important indicator of ante-mortem burns in comparison of presence of soot in lower respiratory tract.

Methods: In this study 47 charred bodies that referred to legal medicine organization of Tehran, Iran from 2005 to 2006 were evaluated. In according to history, autopsy findings and penal records bodies were divided to 3 groups: 1-Definite ante-mortem burns (19 cases) 2-Definite post-mortem burns (14 cases) 3-Undetermined burns (14 cases). Each group was evaluated for carboxyhemoglobin level by Spectrophotometer and presence of soot in lower respiratory tract. Carboxyhemogolobin levels above 15% were interpreted as positive.

Results: From 47 bodies, 44 cases (93.6%) were male and 3 cases were female. The mean (SD) of age was 28.3 (9.6) year old. Carboxyhemoglobin was positive in 42.1% of cases in group 1, none of the group 2 and 14.3% group 3. Soot was seen in lower respiratory tract of all of bodies in group 1, none of the cases in group 2 and 57.1% of group 3.
Conclusions: As the carboxyhemoglobin was positive in some of the ante-mortem burns and was negative in all of the post-mortem burns, we can result that carboxyhemoglobin can be used as a definite finding for ante-mortem burns, but negative carboxyhemoglobin doesn't mean a post-mortem burn. Therefore for distinguish ante-mortem from post-mortem burns we should use other findings beside of carboxyhemoglobin.
Keywords: carboxyhemoglobin, soot, charred bodies

P35. Relationship between postmortem ethanol level in femoral blood and vitreous humor

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Introduction: Determination of post mortem ethanol is one of the most frequency requested analysis in forensic toxicology, but many factors can influence evaluation of postmortem ethanol concentration e.g. unavailability of blood samples for toxicological analysis or contaminated samples, so there is an enormous effort to find alternative sampling sites such as vitreous humor. Quantitative alcohol determination in a variety of postmortem specimens may provide important interpretive information. The main purpose of this study was to establish correlation between ethanol concentration in femoral venous blood (FVB) and vitreous humor (VH).
Methods: Ethanol concentrations were determined in specimens of FVB and VH obtained from 500 forensic necropsies. The specimens were analysis in duplicate by headspace gas chromatography (HS-GC). The limit of detection (LOD) of ethanol in this study was 3mg/dL.
Results: In 71 instances, ethanol was present in FVB, whereas the VH ethanol concentration was reported as negative. These cases were excluded from statistical analysis. The concentration of FVB ethanol was higher than VH in 97 specimens and in the other specimens VH was higher than FVB. Ethanol was present in FVB at a mean ±SD concentration of 121.73±60.40, compared with 133.07±63.06 in VH. The mean ±SD of VH/FVB ratio of ethanol was 1.122±0.214. Pearson's correlation coefficient between them was 0.936. At ethanol concentration ≤100 correlation coefficient was 0.737 and in concentration above 100mg/dL this value was 0.88
Conclusion: There is a good correlation coefficient between vitreous humor and femoral venous blood in determination of postmortem alcohol concentration and there is small difference between them, so vitreous humor can be used as an alternative sample to femoral venous blood for alcohol determination.
Keywords: femoral blood, vitreous humor, alcohol concentration

P36. The effect of temperature and preservative on ethanol production in postmortem blood

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Introduction: Blood is the usual specimen provided for ethanol analysis but it appears artificially low or high if there has been inappropriate or inadequate storage of the specimen. This is usually the result of contamination of the specimen by microorganisms which metabolize glucose and other compound in blood to ethanol. The aim of this study is minimizing this problem by collecting with appropriate preservative (eg: NaF) and storage at proper temperature.
Methods: Ethanol concentration was determined in 70 specimens of blood by headspace-GC method and effect of temperature and preservatives were studied on them.
Results: In 70 samples, 84% were male and 16% were female. We found that without preservative, specimens that stored at 4°C for 96h showed an increase in ethanol concentration ranging from 20 to 50 mg/dL (mean ±SD: 34.24±9.20) With addition of 1% sodium fluoride, there was no significant increase in ethanol concentration. At 25°C this same specimens showed an increase ranging from 35 to 65 mg/dL (mean ±SD: 48.40±9.16) and 15-25 mg/dL (mean±SD: 19.43±3.79) increase after addition of 1% sodium fluoride.
Conclusion: We have demonstrated that even at 4°C, significant amount of ethanol can form in the absence of preservative due to activity of microorganisms like *Candida albicans, Escherishia coli*; Additionally we have demonstrated that the addition of NaF to blood prevents the formation of ethanol at storage in 4°C, and it increase 19 mg/dL in 25°C with preservative. Therefore we believe that NaF should be added as a precaution to all samples and storage at 4°C.
Keywords: ethanol, concentration, blood, preservative, temperature

P37. Drug related traumatic deaths in istanbul between 1990 and 2000

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Aims: Turkey, as a historical opium producer, has continuously dealt with abuse and addiction. Strong increases in heroin abuse have also been witnessed in some countries, such as Turkey, that lie along the main drug-trafficking routes. Despite the role of Turkey in drug trafficking, the issue of traumatic, drug related deaths in Turkey has not been properly investigated. The objective of this study was to identify characteristics of drug related traumatic deaths in Istanbul between 1990 and 2000.
Methods: This piece of research is a retrospective registry-based descriptive study. All autopsies (26421) that were performed at the Council of Forensic Medicine, Ministry of Justice in Istanbul, between 1990-2000 were reviewed. The cases were classified into eight groups according to the nature of the case: shooting by homicide, shooting by suicide, homicide by asphyxia, suicide by asphyxia, blunt force by homicide, blunt force by accident, force with a sharp object and suicide by jumping.
Results: Victims were mostly male and the mean age was 37 years. In our sample the most common way of suicide was asphyxia with 29 cases. Asphyxia was followed by jumping and shooting, 14% and 9.1% respectively. Shooting by homicide and suicide is common among relatively young people, aged approximately 35 years old whereas suicide by asphyxia and blunt force by homicide cases were more prevelant among those in their forties. In total, the most common cause of death was suicide by asphyxia and accidental blunt force. The most common drugs found in victims were benzodiazepines and heroin. These two drugs had played an important role in almost all the traumatic deaths in our sample.
Conclusion: Benzodiazepines, cannabis and heroin were the most common drugs. Some specific patterns were found for drugs in our sample. While benzodiazepines are common in suicide cases, cannabis was frequent among shooting by homicide cases. Heroin was approximately equally seen on all traumatic deaths. Benzodiazepines and heroin are especially frequent among road traffic accidents.
Keywords: trauma, illicit drug, death

P38. Post mortem isoflurane redistribution following pseudo-therapeutic use

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Introduction: We report the case of a 32year old man who died following the recurrent misuse of isoflurane. He had used isoflurane to aid sleep. We present data on post mortem redistribution, including unique data on post mortem vitreous concentrations of isoflurane.

Method: Femoral blood, urine stomach content, lung tissue, brain and vitreous were obtained at post mortem within 24 hours of death. Samples were immediately stored in air tight containers. These were initially refrigerated at 4ºC for 3 days and then frozen until analysis.All preparations were performed at 4ºC. 200 µL internal standard (from a sealed isoflurane bottle seized by police) was added to 1mL of sample or calibration standard and sealed immediately in a 5 mL head-space vial. Tissue samples were prepared using 1 part tissue to 1 part distilled water and homogenized. The gas chromatograph system consisted of a Varian 3900 fitted with a CP-PoraPLOT® Q capillary column (12.5 m x 0.32 mm ID, 10 µm film thickness; Varian), and automated headspace sampler (CTC Analytics Combi PAL). The carrier gas was nitrogen at a constant flow of 2.5 mL/min. Following incubation for 5 min at 60ºC, 500 µL of sample was automatically injected at 200ºC, operating at a split ratio of 1:10. The oven temperature was programmed starting at 50ºC for 5 min, increased at 20ºC/min to 180ºC and held for a final 0.5 min (total run time 12 min).

Results: The blood, urine and gastric content were also screened for drugs of abuse and other volatiles with no other positive findings. The isoflurane concentrations detected were; femoral blood 25.8 mg/L, urine 16.8 mg/L, gastric content 139 mg/L, lung 25.6 mg/kg, brain 45.5 mg/kg, vitreous 13.5 mg/L

Discussion: The levels of isoflurane in blood, urine, stomach lung and brain are comparable to other cases in which isoflurane misuse has been reported as the mode of death, although the brain concentration is possibly lower than expected. This may be in part due to the retrieval and storage of the brain post mortem. The new data on vitreous levels suggests that vitreous levels may be similar to urine levels.

Keywords: isoflurane, post mortem redistribution, vitreous humour

P39. Fatal tricyclic antidepressant overdose complicated by acute colonic pseudo-obstruction

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Introduction: Tricyclic antidepressants are a class of drugs commonly used for the treatment of depression. These drugs account for approximately 20 to 25 percent of overdoses that require acute medical admission. The most common cause of mortality is linked to cardiovascular toxicity, and morbidities include anticholinergic effects (e.g., ileus, constipation, urinary retention) and respiratory complications.

Case report: A 55-year old female was found in coma at her home and transferred to intensive care unit. She had a history of psychiatric illness, and was treated by intramuscular amitriptyline. Following drugs were found at home: meprobamate-acepromazine, amitriptyline, nordazepam, famotidine, allopurinol, tramadol, oxomemazine, acetylcysteine, pravastatine, lactitol. Abdomen tomodensitometry performed soon showed massive colonic dilatation. Despite surgical treatment, the patient died. The post-mortem examination showed that the death was linked to massive colonic occlusive syndrome, associated with ischemia of the body's lower half consequence of colon- induced aorta compression. Pleural cavity's blood analysis by liquid chromatography/photodiode array detector, gas chromatography/mass spectrometry and gas chromatography/flame ionisation detection showed the presence of amitriptyline, nortriptyline, meprobamate, nordazepam, midazolam and ethanol. Sum of amitriptyline concentration (0.62 mg/L) and nortriptyline concentration (0.45 mg/L) was 1.07 mg/L. This value was higher than the toxic one of 0.5 mg/L, found in the literature. Other drugs were quantified in therapeutic concentration ranges. Pleural cavity's blood ethanol level was 0.35 g/L.

Conclusion: Post-mortem examination and toxicological analysis led to believe that the death was caused by acute colonic pseudo-obstruction (Ogilvie's syndrome) with ischemic complication. This syndrome was probably the consequence of amitriptyline overdose associated with other drugs intake as neuroleptics (oxomemazine, acepromazine) and opioids (tramadol) known to promote Ogilvie's syndrome.

Keywords: tricyclic antidepressant, acute colonic pseudo-obstruction

P40. Involvement of pholcodine in the cause of death in 127 forensic cases in France

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Introduction: The use of pholcodine as a cough suppressant is a typically European phenomenon. Because it is an opiate, there is always a risk of abuse or misuse. In the absence of bibliographical data, a study of forensic cases was carried out among the members of the forensic toxicology committee of the SFTA to evaluate potentially toxic or lethal values of *postmortem* levels of pholcodine (PH) in blood.

Method: The retrospective study (2000-2006) included the deaths for which PH was present and a cardiac or peripheral blood assay was performed. The following were taken into account: age, gender, circumstances of death, observations at autopsy and blood alcohol and other substances concentrations. The analysis led to the definition of a classification of causes of death, established as independently as possible of the PH concentration.

Results: 10 members submitted a total of 127 useable cases: 89 males and 37 females (1 sex unknown) aged between 2.5 and 94 years (median age 46). The blood was of cardiac (38.1%), peripheral (40.5%) or unknown (21.4%) origin. The following table reports the PH concentrations (PHC) according to cause of death:

Role of PH / cause of death	[categories]	n	Median PH (µg/L)	Min-max PH (µg/L)	PH>200µg/L (%)
PH involvement highly probable - overdose of PH alone or mainly PH	**[1]**	**8**	**1165**	**472-8130**	**100**
PH involvement probable Medication/alcohol overdose:	**[2]**	**40**	**280**	**7-12 118**	**58**
- sufficient to explain death	[2.1]	27	289	7-12118	59
- implying potentiation through PH	[2.2]	13	258	12-1064	54
PH involvement possible + other causes of death	**[3]**	**38**	**392**	**20-6270**	**71**
- death by "trauma" where PH may have modified behaviour or vigilance	[3.1]	22	392	33-3300	68
- presence of predisposition or pathology	[3.2]	16	410	20-6270	75
PH link excluded a priori + other causes of death	**[4]**	**23**	**90**	**5-930**	**26**
Cause of death unknown	**[5]**	**18**	**200**	**2-980**	**50**

The median PH was significantly higher among the deaths due to PH alone **[1]** (Kruskal Wallis; p<0.002). Three deaths in this category **[1]** had a PH of less than 1000 µg/L (472, 832 and 987). Of the cases with a cause of death other than PH **[3 and 4]**, those with possible PH involvement **[3]** presented a significantly higher median PH (p<0.001), and 71% had a PH higher than 200 µg/L. Of the deaths in theory not linked with PH **[4]**, 26% had a PH higher than 200 µg/L, with 2 reaching values of 810 and 930 µg/L. All the laboratories participated with success to a experimental proficiency test (PH in whole blood). Nonetheless, this study keeps some weaknesses: bias in recruitment and classification.

Conclusion: These results suggest the possible involvement of PH in the cause of death when the PH exceeds 200 µg/L. In addition, the eight cases of death with PH alone **[1]** show that, in theory, a PH higher than 470 µg/L is compatible with death due to PH toxicity. These preliminary results imply the need for routine PH assays and indicate that a multicentre prospective study would be useful.

Keywords: pholcodine, cause of death, *postmortem* toxicology

P41. Unusual concentrations of cocaine and metabolites in a shooting incident

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Introduction: In this paper, we present the case of a 32 year-old man who was shot while parking his car. At autopsy, death could be explained by loss of blood and tissue damage because of multiple shot injuries. Toxicological analysis on alcohol and drugs of abuse was performed to investigate whether the behavior or consciousness of this man was influenced by the presence of alcohol, drugs and/or benzodiazepines at the time of the incident.

Methods: Concentrations of alcohol were determined in whole blood (with NaF as preservative) and urine (with NaF as preservative) by head-space GC with flame ionization detection. Whole heart blood was screened for the following classes of drugs by using ELISA: amphetamines, benzodiazepines, cannabinoids, cocaine-metabolite, methadone, methamphetamines and opiates. The presence of cocaine and metabolites was confirmed in femoral blood (with and without preservative), heart blood, urine and brain tissue using GC-MS after SPE and derivatization.

Results: In blood and urine alcohol concentrations of 0.69 and 0.99 mg/mL (g/L) were measured. Using ELISA, we found an indication for the presence of cocaine-metabolite in heart blood. GC-MS analysis in femoral blood gave the following results: cocaine 0.12 mg/L, benzoylecgonine 0.01 mg/L and methylecgonine 0.04 mg/L. Because of the unusually low concentration of benzoylecgonine, the analysis in femoral blood was repeated and other biological samples were tested as well. The results are presented in Table 1. The results show that cocaine is present in active concentrations in all samples while benzoylecgonine and methylecgonine are absent or present in very low concentrations.

Table 1. Concentrations (blood and urine in mg/L; brain tissue in mg/kg) of cocaine, benzoylecgonine and methylecgonine in biological matrices.

Matrix	Cocaine	BE	ME
Femoral blood (second analysis)	0.14	nd	0.04
Femoral blood (with NaF)	0.16	0.02	0.02
Heart blood	0.18	nd	0.12
Urine	0.50	0.06	0.04
Brain tissue	0.12	nd	0.03

nd=not detectable

Conclusion: The results showed the presence of alcohol and cocaine in blood which might have influenced the behavior or consciousness of the deceased at time of death. In the literature, explanations were sought for the absence or very low concentrations of benzoylecgonine in blood and brain tissue. The only plausible explanation is very recent intake of cocaine.

Keywords: cocaine, metabolites, postmortem

P42. Evidence provided by LC-MS of forced ingestion of faeces in a 4-year-old child

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Introduction: A 4-year-old child, with a thin corpulence was found in a state of apparent death at the residence of his mother. Death was the consequence of a cranio-facial and cerebral trauma and digestive internal bleeding. Moreover, it was found during the autopsy the presence of several bruises, traumatic lesions and burns on the whole of the body as well as brown and malodorous spots, evoking excrement, over the trunk, in the hair, but also in the mouth, the oesophagus and even of the respiratory tracts. It was hypothesised that these residues came from the forced ingestion of the stools of the family dog or the child himself and could have taken part in the death by mechanical asphyxiation. In order to argue the barbarian character of the acts inflicted on the child, we were asked in addition to the usual analyses to prove in a scientific way that these spots were indeed excrements. We put forth the hypothesis that the best markers of the faeces were biliary pigments and porphyrins (especially the stercobilin and urobilin), and undertook their characterization by LC-MS.

Methods: The samples to be analyzed were taken using swabs during the autopsy. We used as positive controls the faeces of various patients sampled under similar conditions. The negative controls corresponded to blood and urines spots. The cotton tip of the swabs were placed in 1 mL of methanol. After homogenisation and filtration, the solvent was evaporated. The dry residue was reconstituted with 30 µL of methanol and 10 µL were then injected into a HPLC system (Applied Biosystems 140B). The chromatography was carried out over 15 min using a NovaPak C18 column (150x2.0 mm, 4 µm) and the mobile phase consisted of a methanol - ammonium formate buffer gradient (2 mmol/L pH 3). The detector (API 100, PE Sciex) was used in positive ionization mode (ESI+) in SIM with m/z=595.5+343 and 591.5+470 for the stercobilin and urobilin, respectively.

Results: The stercobiline was elute in 11.3 min and urobilin in 11.0 min. The results were given as presence or absence of porphyrins, a presence of stercobilin signaling the presence of faeces.

Sample	Swab coloration	Stercobilin (ion m/z 595.5)	Stercobilin conclusion	Urobiline (ion m/z 343)	Urobilin conclusion
hair	+++	608 140	Presence	12 520	Presence
mouth	++++	2 579 970	Presence	149 640	Presence
trachea	+	8 360	Presence	-	Absence
thorax	++	279 910	Presence	2 140	Presence
oesophagus	+	6 530	Presence	-	Absence
anus	++++	844 450	Presence	2 990	Presence
thigh	++	190 670	Presence	2 350	Presence
blood	+++	-	Absence	-	Absence
urine	++	-	Absence	254 780	Presence

Conclusion: The presence of stercobilin (most often associated with urobilin) in the samples showed without ambiguity the presence of faecal

material in hair, oral cavity, trachea, thorax, oesophagus, anus and on the thigh. The analysis by LC-MS (or genetic analysis) did not make it possible to determine the human or animal origin of these excrements, but remains reliable in forensic cases where search for faeces is necessary.

Keywords: porphyrins, mass spectrometry, child maltreatment

P43. Smoking cessation can be fatal! A case report involving varenicline

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Introduction: Tobacco use claims more than 440,000 lives each year, making it one of the leading causes of mortality in the world. The most effective smoking cessation programs involve a combination of pharmacotherapy (nicotine replacement therapy, e.g. bupropion) and behavioral and/or cognitive counselling to improve abstinence rates. Varenicline (Champix in France and the UK), the most recent agent approved for tobacco cessation, is the first drug in a new class (alpha4beta2 partial agonist) that binds to the nicotinic receptors to release dopamine and alleviate withdrawal symptoms. As the literature reports suicidal ideation being linked to varenicline as an issue, we present here the suicide of a man, under therapy of the drug.

Method: The deceased (a 39 year-old man) was found on the landing at his home address with slash wounds to his wrist. Life was confirmed to be extinct in situ by the attending Paramedic. The deceased had been prescribed varenicline for several months at a dose of 1 tablet (1 milligram) twice daily. The lab received a 12 mL blood sample to develop a procedure to test for varenicline and document the case. Due to its selectivity and sensitivity, LC-MS/MS was chosen as the best approach. 1 mL of blood was extracted with 5 mL of a mixture of dichloromethane/isopropanol/n-heptane (25/10/65) at pH 9.5 (phosphate buffer), in the presence of diazepam-d_5, used as an internal standard (IS). The resultant blood extract was separated on a XTerra MS C18 column using a gradient of acetonitrile and formic acid in water. Drugs were identified by 3 or 2 transitions (m/z 212>169, 212>183 and 212>195 and 290>154 and 290>198 for varenicline and IS, respectively). LOQ of varenicline was 1 ng/mL.

Results: Varenicline was extracted according to our general procedure for drug screening. There was no chemical specificity of the compound. The concentration of varenicline in the blood was determined to be 10 ng/mL. This concentration could not be compared with therapeutic levels, as there are no therapeutic concentrations reported in the literature. However, when considered alongside the manufacturers stated volume of distribution for the drug (415 L, CV 50%) and the normal dosage of 1 milligram it is unlikely to represent an excessive dose. Although there is no definite warnings about potential psychiatric problems, varenicline has been considered an unsuitable treatment for subjects known to have mental disorders, while reports about varenicline-induced manic episodes and exacerbation of schizophrenia have been published in the literature. In December 2007 the European Medicines Agency issued a press release in which they concluded that updated warnings about the risk of suicidal ideation should be made alerting doctors and patients of the potential for such occurrences.

Conclusion: A case involving the suicide of a 39 year-old man under varenicline therapy is presented. Due to its potential effects on behavior, the influence of the drug on the mental functioning of the user should be considered in cases of suicide.

Keywords: varenicline, post mortem, suicide, LC-MS/MS

P44. Buprenorphine/Norbuprenorphine identification in putrefied biological samples: evaluation of a buprenorphine related death

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Introduction: Deaths related to buprenorphine are rarely discovered, in Italy, even though this drug has been widely using, since 2000, both as analgesics for severe pain and as substitution treatment for heroin addicts, instead of methadone. This paper describes a case report where buprenorphine/norbuprenorphine are identified and quantified (in GC/MS) in putrefied biological samples, in order to demonstrate the persistence of Bup/Norbup in post mortem samples and highlight the incidence of buprenorphine in deaths involving heroin and other psychotropic drugs addicts.

Case report: A young Marine (USA) was found dead, in his residence, in an advanced state of decomposition. At inspection of the death scene were found the following pharmaceutical blisters: Subutex (8 mg), Minias (2,5 mg/mL), Lormetazepam (2,5 mg/mL), Zolpidem, Sedatol. There were neither syringes nor violence evidences. Because putrefaction, autopsy not revealed typical signs of acute narcotism, such as cyanosis, pulmonary oedema, visceral congestion. Furthermore liver, blood, hair were collected. Moreover the few liquids from the bladder and the gall-bladder were taken washing them by physiological water.

Materials and methods: Bup/Norbup were extracted from putrefied samples (blood, liver, urine and bile) and hair, with Toxi- Lab Tubes™ "A", after acid hydrolysis for hair and enzymatic hydrolysis (β-glucuronidase from *Helix pomatia*) for other biological samples. Instrumental identification was carried out on (5890/5973N) Agilent GC-MS (SIM mode), monitoring respectively 450-482-506 ion for buprenorphine and 468-500-524 ion for Norbup, compared to certified materials (blood and urine from Medichem, Germany) and deuterated internal standards.

Results: In spite of the advanced putrefaction and dilution (as regards of urine and bile) the presence of Bup/Norbup was demonstrated in each biological samples (liver 8,4 ng/g; blood 24 ng/mL; urine and bile positives). Complementary systematic analysis in GC-MS, revealed Clozapine, Lormetazepam and Amoxapine, only in urine and bile. Hair results obtained from proximal and distal segments, showed in Tab. I, define a chronic drug abuser and suggest a decrease in heroin and cocaine intake. These data support the hypothesis that the subject was under Bup substitution treatment with contemporary use of antidepressive and tranquillizer drugs.

Table I. Buprenorphine and other drugs in hair.

Hair	Bup (ng/mg)	Norbup (ng/mg)	Cocaine ng/mg	BEC ng/mg	6-MAM ng/mg	Morphine ng/mg
Proximal (0–3 cm)	12,7	5,9	21,87	2,73	102,18	24,32
Distal (3–6 cm)	49,8	<LOD	69,42	5,30	320,15	78,65

Conclusion: Our data confirm that putrefaction doesn't change persistence of Bup/Norbup in biological materials nor is a relevant interference in identification and dosage of this drugs.

Keywords: buprenorphine, putrefied biological samples, GC-MS

P45. Three ethylene glycol poisoning cases

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Introduction: Ethylene glycol is an achromatic odourless substance from toxic alcohol family, used in antifreeze solution, cosmetics and some dyes. Poisoning with this material is almost by oral consumption. The diagnosis

is concluded from medical history, clinical manifestations and laboratory tests. Therapy include, sodium bicarbonate, ethanol and hemodialysis have special importance. This study consisted of case reports on ethylene glycol poisoning and diagnostic-therapeutic suggestions from this intoxication.

Methods: We report three cases of ethylene glycol poisoning in males of 18,22 and 28 years old, which were intoxicated by oral unaware usage of antifreeze solution and referred to poisoning emergency ward of Loghman hospital.

Results: Emergency diagnosis and following suitable and rapid treatment are very important. Blood samples were collected. But regarding to the lack of facilities in our center, it was impossible to obtain the ethylene glycol quickly. Because medical histories of these three cases were consistent with oral consumption, of antifreeze solution over 250 mL (lethal dose for ethylene glycol); rapid diagnosis and treatment have a special importance. The clinical manifestations were drunkenness without breath alcohol odour and then dizziness, weakness, vertigo and diplopia. All this findings indicated the high probability of ethylene glycol intoxication so rapid treatment should be initiated. Metabolic acidosis was concluded in the three cases, it was severe in one case. A few days later ethylene glycol intoxication was confirmed with 20 mg/dL, 40 mg/dL and 32 mg/dL blood levels. Treatment with sodium bicarbonate (to improve metabolic acidosis) and ethanol (in order to prevent glycolic production) began immediately. Hemodialysis was performed as well. Characteristic paraclinical and clinical manifestations improved quickly after hemodialysis.

Conclusion: Regarding severity of intoxication in these three cases and very special importance of rapid diagnosis and lack of facilities in our center, medical history and clinical manifestations were sufficient for primary diagnosis and begin treatment. Hemodialysis has to be considered as the first choice as soon as possible.

Keyword: ethylene glycol, intoxication

P46. Suicides by the mercury

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Introduction: For intentional intoxication, inorganic substances are nowadays used only seldom. In spite of that, we meet them in our toxicological practice. In this case, intoxication with mercury is described either with elementary mercury or its salts.

Cases: 39-year old man was found in his home wet with vomits. Chemical burn of lips and mouth, metabolic acidosis**,** anuria and shock state were found on the body. After six hours of resuscitation the man dies. Colagulative necrosis on the tissue of gullet and the gastric tissue looking like being affected by hemorrhagic gastritis were found during autopsy. Sublimate (mercuric chloride) was proved in the tissue of GIT. A 25-year old man is treated for multiple suicidal attempts (intoxication by medicaments). He also undergoes psychiatric treatment as being a gambler. In 2005 he applicated metallic mercury intramuscularly in armpit. In the end of 2006, he applicated metallic mercury s.c. (subcutaneous) under the clavicle. The man lives without health troubles. X-ray showed applicated metallic mercury. A 34-year old man, user of cannabis, methamphetamine and ecstasy, commited suicidal attempt, using flunitrazepam and cutting his veins. In 2006 he commited another suicidal attempt by metallic mercury. He applicated mercury i.v. (intravenous) from 2 thermometers and swallowed mercury from 1 thermometer. X-ray photographs and CT scan show diffusion of the metallic mercury in his body (lungs, heart and brain). The man lives without health troubles so far.

Method: A method for the determination of total mercury in urine and blood using trace mercury analyzer AMA-254 was developed. Samples of urine or blood was ashed in furnace of analyzer AMA-254 (Altec Praha); mercury was determinated directly without sample pretreatment. A single-purpose atomic absorption spectrometer AMA-254 used the technology of mercury vapours generation with following enrichment in gold amalgamator. Parameters of analysis: drying - 250 sec, decomposition: 200 sec, waiting: 80 sec. 0,1 mg/L of mercury in the blood and 0,5 mg/L of mecrury in urine was deteminated using the method of AAS.

Conclusion: Toxic effects of compounds of mercury and fumes of mercury are well described and elucidated. Effect of metallic mercury applicated i.v. into human body is different in every case. In this case, organism eliminates mercury without any apparent toxic effects on subject.

Keywords: mercury, suicide, CT scan

P47. A case of fatal intoxication with documented isopropyl alcohol concentrations in postmortem tissues

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Introduction: Isopropyl alcohol (IPA) is a volatile, constituent of commonly used household solvents. IPA intoxications can be lethal by themselves, or in combination with other central nervous system depressants. We report here a documented case of fatal intoxication with IPA concentrations identified in post mortem tissues. To our knowledge, no such data exists in literature. A 12 years old girl from a disadvantaged family setting went to a party where a lot of unidentified brewages or solutions were abused (drinking and/or sniffing) by the participants. The following morning, she presented a gastroenteritis syndrome with cephalgia. Due to her declining health condition in spite of acetaminophen self-treatment, she was admitted in the emergency unit about 30 hours after the party. On admission, she presented consciousness disorder and in spite of an adequate treatment in the intensive care unit, her status quickly deteriorated with severe cardio-vascular failure and coma resulting in death. The deterioration occurred 6 hours after her admission. Autopsy findings showed bilateral pneumonia with fatal massive pulmonary oedema and suspected sepsis. The collected biological samples (urines, vitreous humour, gastric content, bile, liver, brain, lung), without a blood sample, were sent to the laboratory for forensic toxicological analysis.

Methods: A large screening of drugs and toxics compounds in both urines and gastric content was performed using high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD), gas chromatography - mass spectrometry (GC-MS) and liquid chromatography-electrospray-tandem mass spectrometry (LC-ES-/MSMS). More selective analysis for several classes of drugs of abuse were carried out with various ad-hoc methods using HPLC-DAD, LC-ES-/MSMS and GC with various detection modes. In particular, a headspace (HS) technique coupled with GC/MS technique dedicated for volatile substances investigations was performed for these substances (and its metabolites) in urine, bile and gastric content.

Results: The main analytical finding was the presence of isopropanol and acetone in various tissues as presented in the following table:

	Urine (mg/L)	Gastric content (mg/L)	Liver (µg/g)	Brain (µg/g)	Lungs
IPA	8.3	21.7	52.6	4.8	detected
Acetone	631	37.9	13.2	36.3	detected

Conclusion: The data analysis is hindered owing to the absence of blood concentration values and to the delay (30 hours) between the abuse and the medical care as IPA elimination is fast. Nevertheless, our toxicological findings are consistent with the acetone odour of the corpse noticed at autopsy. Furthermore, IPA toxicity can explain the pneumonia and the subsequent sepsis observed at autopsy as the result of the lung alterations. According to the long time delay between IPA intake and the death (about 36 hours),

the observed urine concentration of IPA and its main metabolite, acetone, are comparable to those of similar intoxication cases found in literature. The present fatality was finally attributed to a massive volatile substance abuse practice including IPA drinking and/or sniffing abuse.

Keywords: isopropyl alcohol, HS-GC/MS, fatality

P48. Ketamine testing within a large-scale workplace drug testing programme

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Introduction: Ketamine has become a regularly encountered drug of abuse within the UK club scene. It was added to the UK's scheduled list of controlled drugs in January 2006. A recent UK Governmental study has identified a self-reported level of ketamine use in the general population of 0.3% within the last year, and of 0.8% for 18-24 year olds. These levels are approximately one eighth of the level of self-reported cocaine use quoted in the same study.

The UK Ministry of Defence (MoD) operates a compulsory drug testing programme for UK military personnel, involving some 125,000 urine samples per year being collected and tested for evidence of abuse of controlled drugs. Overall positive rates in the MoD programme are currently around 0.7%, significantly below the levels seen in most other workplace drug testing programmes.

Once ketamine became a controlled drug, it could be included as a target analyte within the MoD programme. However, for large scale testing, a suitable immunoassay for ketamine was required. When this became available, ketamine testing was added to the military testing regime, initially on an experimental basis, to help to establish the level of prevalence amongst military personnel and to identify any practical and analytical issues.

Methods: Analysis is carried out using immunoassay screening, using Microgenics reagents and Olympus autoanalysers, followed by confirmatory testing of potentially positive samples using LC-MS-MS. Confirmatory testing is carried out by identification of ketamine and norketamine, following hydrolysis and extraction from the urine matrix using solid phase extraction. The method was validated by spiking blank urine with Cerilliant reference materials, and recoveries of >80% were achieved. Screening was commenced with a cut-off level of 100 ng/mL, however this was found to result in an excessive proportion of samples being identified for confirmatory testing and which then proved to be negative. The screening cut-off was reviewed and raised to 250 ng/mL. A confirmatory cut-off level of 50 ng/mL of ketamine and norketamine was applied.

Results: Analysis of an initial batch of approximately 20,000 samples produced 4 samples which were positive above the confirmatory cut-off level for both ketamine and norketamine. Three further samples contained traces of ketamine and norketamine below the confirmatory cut-off level.

Conclusion: Large scale screening for ketamine within workplace drug testing programmes has been demonstrated to be feasible using a commercial immunoassay with a screening cut-off level of 250 ng/mL and LC-MS-MS confirmation using a confirmatory cut-off level of 50 ng/nl for ketamine and norketamine. In positive samples, an associated, and more significant, peak was observable at confirmation, which is believed to be dehydronorketamine. A reference material for this substance has been obtained, and, if the identification of the third peak is confirmed, dehydronorketamine appears to represent a more sensitive target species for confirmatory analysis.

Keywords: ketamine, workplace drug testing

P49. Rapid simultaneous determination method for fenitrothion compounds and their metabolites in human serum and urine by LC–MS

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Introduction: Fenitrothion, one of the organophosphorus pesticide, is extensively used in many countries and its poisoning is often fatal and life threatening. The aim of the study is to develop a simple and rapid method by LC-MS for fenitrothion, its metabolite (3-methyl-4-nitrophenol), and 5 other organophosphorus pesticides that are frequently included in fenitrothion compounds (acephate, cyanophos, methidathion, ethyl p-nitrophenyl phenylphosphonothionate, and malathion) in human serum and urine samples.

Methods: Following deproteinization by acetonitrile, an aliquot of the biological sample was injected into an LC system that used a C_{18} column and 10 mM ammonium formate-methanol as the mobile phase.

Results: The LODs ranged from 0.125 to 0.5 μg/mL in serum and 0.25 to 0.75 μg/mL in urine. Excellent linearity was observed for the LOQs: up to 20 μg/mL in both serum and urine samples. Extraction recoveries were satisfactory and ranged between 60.4% and 104.9% in serum and between 59.4% and 110.3% in urine. Intra- and inter-assay precision and accuracy were satisfactory for most of the pesticides analyzed. In terms of temperature stability, of all the organophosphorus compounds analyzed, acephate and malathion exhibited the most rapid degradations over 24 h at room temperature and over 3 d at 4°C. However, on frozen preservation, all samples remained relatively stable throughout the entire 4-week testing period. The present method was successfully applied to an actual poisoning case who ingested fenitrothion and acephate emulsion. The determined serum concentrations of 3-methyl-4-nitrophenol, acephate, and fenitrothion were 1.02, 7.1, and 0.72 μg/mL, respectively. The determined 10% diluted urine concentrations of 3-methyl-4-nitrophenol and acephate were 17.4 and 13.4 μg/mL, respectively; fenitrothion was not detected.

Conclusion: This method is simple, accurate, and useful for the determination of organophosphorus pesticides, including fenitrothion and its metabolites, and should benefit both clinical and forensic toxicology.

Keywords: fenitrothion; LC-APCI-MS; validation

P50. Artifactual production of cyanide from coffee and tea drinks in the presence of nitrite

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Aims: In unknown poisoning cases, forensic toxicologists is required to elucidate the cause of death, and one of the most noticeable poisons is "cyanide". Previously, we found that cyanide was artifactually produced from coffee drinks. In this presentation, we report that cyanide is nonspecifically produced from polyphenol compounds in the presence of nitrite.

Methods: Cyanide was measured by head-space gas chromatography (HS-GC) (column, GS-Q (0.53 mm x 30 m); column temperature, 140ºC; helium flow rate, 5 mL/min; split ratio, 5:1; detector, nitrogen phosphorus detector). Various polyphenol compounds were mixed with sodium nitrite in 8.7 mL glass vial (final liquid phase: 1 mL) under acidic conditions of 10% phosphoric acid or 0.6 M sodium phosphate buffer (pH 5.0), and after 30 min incubation at 50ºC for 30 min, the resulting 0.5 ml of the head-space was injected into the gas chromatograph.

Results: Hydrogen cyanide peak was eluted at 2.2 min, and the peak area was converted for measuring the cyanide concentration in the liquid reaction mixture. From the 50ºC incubation mixture of the commercial instant coffee drink (Nescafe Goldbrend Original, 0.15%) and nitrite (50 mM), 1.2 and 0.4 μg/mL cyanide was produced in the presence of 10% phosphoric acid and pH 5.0 buffer, respectively. Cyanide was also produced from the reaction

mixture of nitrite and authentic polyphenol compounds (0.75 µM) such as chlorogenic acid (2.8 and 0.5 µg/mL cyanide production) and gallic acid (1.7 and 0.6 µg/mL cyanide production), but not from the monophenol mixture. Production of cyanide was also ascertained by the pyridine-pyrazolone colorimetric reaction. The produced amount of cyanide was dependent on the substrate concentrations of nitrite and polyphenol, pH, temperature and incubation time.

Conclusion: Nitrite was reacted with polyphenol compounds under acidic conditions, producing significant level of cyanide. Forensic toxicologists should pay attention to this type of the artifactual cyanide production.

Keywords: cyanide, false positive detection, polyphenol

P51. Lindane toxicity in a 52-year-old man

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Introduction: Lindane is an organochlorine pesticide substance found in certain prescriptions in such form as shampoos and topical lotions used to treat pediculosis**.** It is also used as a kind of insecticide substance in meat-packing centers in a gas form**.** Chronic exposure may cause to complications.

Method: A 52- year- old man was referred to Legal Medicine Organization and disability evaluating commission in order to changing his job or being retired. He was working in a meat-packing factory as a worker who sprays Lindane as an insecticide for 20 years. Now he was referred because of neurologic and hepatic symptoms.

Results: In multiple surveys and by rule out of other causing factors it seems that being encountered with this substance chronically caused neurologic and hepatic signs and symptoms such as Tonic - Clonic seizure attacks and hepatic cirrhosis. Liver scan revealed parenchymal liver disease with possibility of presence of space occupying lesion (regenerative nodules…). As lab tests for detecting HBV, HCV, auto immune hepatitis and also Wilson and hemochromatosis all were negative; it seems that this cryptogenic cirrhosis was due to chronic Lindane exposure. EEG shows scattered bilateral sharp waves and in examination psychomotor slowing is observed.

Conclusion: Studies demonstrated that chronic Lindane toxicity may cause neurological and hepatic signs as well as its acute form. Therefore its chronic exposure should be limited.

Keywords: lindane, toxicity, chronic poisoning

P52. Reactivity of paraquat with sodium salicylate: formation of stable complexes

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Introduction: The non-selective contact herbicide paraquat (PQ) has been the cause of thousands of deaths from both accidental and voluntary ingestion. The main target organ for PQ toxicity is the lung. This is mainly due to a characteristic polyamine uptake system in this organ for which PQ is a preferential substrate and due to its capacity to generate redox-cycle. We have recently showed that sodium salicylate (NaSAL) could represent a powerful antidote to be used against paraquat (PQ) poisonings since full survival was observed in preclinical studies, the protection being mediated mainly by an effective inhibition of pro-inflammatory factors such as nuclear factor (NF)-κB, by scavenging reactive oxygen species (ROS), and also through the inhibition of myeloperoxidase activity and inhibition of platelet aggregation. A subsequent study reinforced the potential use of this interesting molecule in the protection against PQ-induced lung apoptosis. With this work we are explaining, by chemistry, the protection conferred by NaSAL that led to full survival of PQ exposed animals.

Methods and results: It is showed for the first time that PQ and NaSAL reacts immediately in aqueous medium and within 2-3 minutes in the solid state. Photographs and scanning photomicrographs launched the hypothesis that a new chemical entity is formed when both compounds are mixed. This assumption was corroborated by the evaluation of the melting point, and through several analytical techniques. Ultraviolet/Visible, nuclear magnetic resonance, gas and liquid chromatography/mass spectrometry/mass spectrometry and infrared spectroscopy revealed that a different chemical compound is formed when PQ is made react with NaSAL, with new peaks appearing or suffering shifts of the maximum of absorption to higher wavelengths. With liquid chromatography/electrospray ionization/mass spectrometry/mass spectrometry, it was possible to obtain the stoichiometry of the charge-transfer complexes. In order to increase resolution, single value decomposition acting as a filter, showed that the charge-transfer complexes with m/z of 483, 643 and 803 correspond to the pseudo-molecular ions, respectively 1:2, 1:3 and 1:4 (PQ:NaSAL).

Conclusion: These results strongly support the importance of using salicylates in the treatment of PQ poisonings.

Keywords: paraquat, sodium salicylate, charge-transfer complexes.

P53. Legal limits in DUID cases. Do we need back-extrapolation in case of delayed sampling?

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Introduction: Impairment approach, *per se* approach with a science-based finite limit, and *per se* approach with a zero limit for a tolerable concentration of delta-9-tetrahydrocannabinol (THC) are the current approaches in driving under the influence of cannabis (DUIC) laws. Determination of the concentration of THC in blood is crucial, regardless of the approach. Since a potentially important time period could occur between the offence and blood sampling, we evaluate the need of back-extrapolation for the calculation of blood THC concentration at the time of the offense. In comparison, a similar evaluation is realized for ethanol according to Swiss rules.

Methods: Among the drivers suspected of driving under the influence of drugs in the Swiss Cantons of Vaud, Jura and Fribourg from 2004 to 2007 (N=1909), a group of drivers suspected of DUIC (N=1079) and a group of drivers suspected of driving under the influence of ethanol (DUIE) (N=964) were considered. Both groups were divided into 6 subgroups relating to the time period between the offence and blood sampling (0-1, 1-2, 2-3, 3-4, 4-6 and >6 hours). THC was quantified in whole blood by GC-MS, and blood ethanol concentration (BEC) was determined by Head-Space-GC-FID. Swiss rules were used for calculation of blood ethanol concentration at the time of the offence by back-extrapolation (absorption period: 2 h, rate of elimination of ethanol: 0.1 g/kg/h).

Results: Samples were obtained principally within the 3 hours following the offence (84% for DUIC and 83% for DUIE). A decrease of the mean values of THC concentrations and a decrease of the mean values of BEC were observed when the time period between the offence and blood sampling increased (10.0, 6.4, 5.0, 4.5, 3.5, 3.1 ng/mL for THC and 1.35, 1.28, 1.23, 1.12, 0.93, 0.73 g/kg for ethanol, respectively). If back-extrapolation calculation is applied for ethanol cases, mean values of BEC remained pretty constant over the different time periods (1.35, 1.28, 1.27, 1.26, 1.20, 1.45 g/kg, respectively). If we applied a per se approach with an analytical whole blood limit of 2.2 ng/mL for THC (Swiss law: limit of 1.5 ng/mL with an interval of confidence of ±30%), a decrease of the number of positive cases was observed

when the time period between the offence and blood sampling increased (68%, 62%, 54%, 54%, 54%, 22%). Similar observation was done if we applied a per se approach with a science-based finite whole blood limit of 3.5 ng/mL (54%, 46%, 36%, 35%, 25%, 12%), as proposed recently by Grotenhermen et al. [Addiction. 2007; 102: 1910-1917]. Those results can be easily understood if the pharmacokinetic of THC and ethanol are considered. Moreover, in case of an important delay of time between the offence and blood sampling we suggest that an impairment approach or a per se approach with back-extrapolation should be used, even if pharmacokinetic of THC is very complex.

Conclusion: *Per se* approach in DUIC is certainly a good pragmatic solution for a majority of cases, in particular if the delay of time between the offence and blood sampling is reasonably short, for example <3 hours when a 2.2 ng/mL positivity threshold is used. If the delay is too important, an impairment approach, a *per se* approach with back-extrapolation, or simply lowering the threshold should rather be used, after evaluation and validation.

Keywords: Driving, cannabis, THC, back-extrapolation

P54. An evaluation of 12-months outcome of drug use probationers in Turkey

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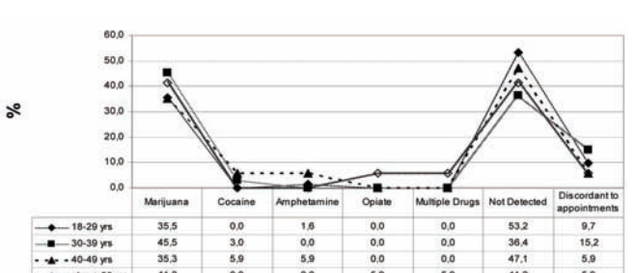
Introduction: The widespread problems dependent on illicit drug use, provocated the new legislative approaches in Turkey. In 5272 numbered Turkish Penalty Code's 109th paragraph it is suggested that probation measures which is related to "The evaluation according to a psychiatrist *clinical diagnosis* with *laboratory findings* for therapies of narcotic, stimulant or inhalant substances and alcohol addiction" put into practice in June 2005.

Methods: This report evaluates the 12-month outcomes of probationers (n=129) who received by Ege University Drug Abuse and Addiction Center. The recorded details of probationer's drug use, familial characteristics and sociodemographic backgrounds. Urine specimens were screened for drugs using CEDIA and positive cases were confirmed by the LC-ESI-MS.

Results: The majority of the subjects were male (98.2%). Eighty percent of the sample was primary school educated. Approximately half of the samples were married (51.2%). Thirty percent of the subjects have insufficient economical level. Ninety-seven percent of the subjects were probationer because of cannabis use/misuse. Alcohol use/misuse was reported by 64.3% of the subjects. The family history of alcoholism was reported by 61.9% of the sample. The marijuana using was decreased from 97.6% to 38.8% during the probation period. In the drug user which has family history positive for drug use, drug use prevalence was 64.7%. For family history negative drug users, this prevalence was 34.3%. The difference was statistically important (Pearson Chi-square test, p=0.01).

Conclusion: This first execution of probation for drug users in Turkey shows that marijuana was the most used illicit drug, coercive treatment and toxicological analysis was effective at least 46.5% on probationers.

Keywords: illicit drug, probation, toxicological analysis



	Marijuana	Cocaine	Amphetamine	Opiate	Multiple Drugs	Not Detected	Discordant to appointments
18-29 yrs	35,5	0,0	1,6	0,0	0,0	53,2	9,7
30-39 yrs	45,5	3,0	0,0	0,0	0,0	36,4	15,2
40-49 yrs	35,3	5,9	5,9	0,0	0,0	47,1	5,9
above 50 yrs	41,2	0,0	0,0	5,9	5,9	41,2	5,9

P55. Adulterated honey raises more than just questions!

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Introduction: Herbal remedies for prophylaxis and treatment of a variety of ailments have been sought after and used for centuries. Many Internet sites promote and sell "safe" and "100% natural" alternatives to prescription anti-impotence drugs such as sildenafil (Viagra®). However, some herbal products for the treatment of erectile dysfunction have been found to contain sildenafil and tadalafil (Cialis®). The case described here involves the promotion of honey claimed to be of herbal origin and sold to improve fertility and cause "distinguished sexual activity". The product listed no prescription drugs amongst its ingredients.

Case report: A 50-year-old man was introduced to the herbal honey by a friend. The patient reported good clinical efficacy to his General Practitioner, who became suspicious about the contents of the product. Two jars of honey were sent to the Analytical Unit, St Georges - University of London, UK for toxicological analysis (one used by the patient the other remained sealed). The manufacturer of the honey product was identified as Etumax and a similar product was purchased by us directly from the Etumax website for comparative analysis. Overall, packaging and description of the product were similar to those used by the patient, although sachets were received via the website, instead of a jar. The listed ingredients in the purchased honey were Radix *Eurycoma longifolia* Extract (200mg), Radix *Panax Ginseng* powder (200mg), Bee Larva Powder (200mg) and pure honey (19.4g). No prescription drugs were listed amongst the ingredients of either product.

Methods: Aliquots of the jar honey (~5g), and the entire contents of each of 6 of the 12 sachets (~20g of honey per sachet) were screened by scanning ultra-violet spectrophotometry (UV) and liquid chromatography with tandem mass-spectrometric detection (LC/MS/MS). A standard was prepared by mixing blank honey with deionised water spiked with typical doses of sildenafil, tadalafil, and vardenafil (Levitra®). Diluted standards, blanks and samples were extracted using pH7 buffer into MTBE, evaporated under vacuum, reconstituted in 80% methanol and injected onto the LC system. The precursor ions $[M+H]^+$ monitored for sildenafil, tadalafil and vardenafil were *m/z* 475, 390 and 489 respectively. The following product ions were monitored for sildenafil *m/z* 58, 100, 283, 311; tadalafil *m/z* 268, 204 and vardenafil *m/z* 151 and 312. The sachet contents were quantified by diluting samples (1 in 1,000,000) so they were effectively aqueous solutions, and analysed against aqueous calibrators in the range 10-500ng/mL.

Results: Screening indicated that both jars submitted by the patient and all sachets analysed, were positive for sildenafil. Tadalafil and vardenafil (other phosphodiesterase type 5 inhibitor drugs prescribed for the treatment of impotence) were not detected in any of the honey samples. Quantification of the contents revealed a mean concentration of 59.4mg of sildenafil per sachet (~3mg/g of honey), with a range of 56.9mg to 62.3mg of sildenafil per sachet.

Conclusion: It is concerning to find another product manufactured by Etumax, being sold as a herbal treatment for impotence, containing a prescription only medicine (POM). Typical doses of sildenafil are in the range 50-100mg per day. The dose of sildenafil per sachet of honey would be sufficient to cause a pharmaceutical effect, or could produce a pharmacokinetic interaction with other medicines. Our findings further demonstrate the potential dangers for patients taking "natural" or "herbal" products that are not under the control of a regulatory agency.

Keywords: herbal, honey, sildenafil

P56. Fatal cytisine intoxication and analysis of biological samples with LC-MS/MS

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Introduction: Cytisine is a toxic pyridine-like alkaloid. Due to a structural similarity of the two molecules, it exhibits pharmacological effects similar to nicotine. Plants containing the alkaloid in various concentrations are found in several genera of the *Faboideae* subfamily, including *Laburnum anagyroides* (also known as golden rain/chain), which is a smallish, decorative tree often planted in parks and gardens. In late spring it is covered in longish, bright yellow inflorescences. Seeds develop in pea-like pods in summer and often remain on the tree all winter. All parts of the tree, especially the bark and seeds, contain the toxin cytisine. Fortunately ingestion of laburnum usually causes only gastrointestinal upset. Severe intoxication is rare but may result in neurological symptoms. Effects may appear within 1 h and include a burning sensation in the oropharynx, nausea, vomiting, abdominal pain and diarrhoea. Headache, dizziness, confusion, dilated pupils, clammy skin, tachycardia, pyrexia, dyspnoea and drowsiness are possible successive symptoms. Recovery is usually complete within 12-24 h. Although large doses of cytisine could cause hallucinations, convulsions, respiratory failure, coma and even death, fatal cases of poisoning are extremely rare due to the compound's innate emetic effect. We report a fatal cytisine intoxication in a 20-year old man who, according to his mother, had drunk a tea prepared from plant material.

Method: Biological fluids and tissues were assayed for ethanol and drugs of abuse (acidic, basic, and neutral organic drugs) using routine methods including immunochemical procedures and liquid-liquid as well as solid-phase extraction procedures with further analysis by HPLC-DAD and GC-MS. For analysis of alkaloids in biological specimens we used a LC-ESI-MS/MS procedure.

Results: Cytisine was found in various samples in following concentrations: peripheral blood 2.5 ng/mL; heart blood 0.9 ng/mL; urine 7.2 ng/mL; stomach content 1.4 ng/mL; liver 4.2 ng/g; kidney 4.7 ng/g; bile 6.1 ng/mL; brain 0.3 ng/g. All other tests revealed negative results.

Discussion: By exclusion of other causes of death (morphological and histological examination) a drug intoxication was determined as the cause of death in the present case. To our best knowledge this is the first case of a fatal cytisine intoxication in a human being in which a quantitative analysis of the toxic substance in various biological specimens was performed. As described in pharmacokinetic studies in mice, highest concentrations were measured in liver, kidneys and bile.

Keywords: cytisine, LC-MS/MS, fatality

P57. Prevalence of 4-hydroxybutanoic acid (GHB) in Québec

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Introduction: 4-hydroxybutanoic acid (GHB) is an endogenous compound present in most mammalian tissues. It was first used as an anaesthetic agent and further to stimulate muscle growth, treat narcolepsy or alcohol withdrawal syndrome and as a recreational drug. Since many years GHB or γ-butyrolactone (GBL) use is increasing in the young population and commonly associated in the context of sexual assaults. Positive forensic cases in the province of Québec (Canada) are presented here from 2000 to 2007; they include chemical submission, driving under the influence of drugs and coroner's cases.

Method: The extraction (liquid/liquid or protein precipitation) vary depending on the nature of the sample and extracts analysed by GC/MS (Agilent 5973N).

Results: The analysis of GHB in biological samples is not part of a general drug screen in our laboratory; its determination is done depending mostly on the information provided by the client and the circumstances surrounding the event.

Table 1. Overview of positive cases in different categories.

GHB	Chemical submission	Driving under influence of drugs (DUID)	Coroner cases
Number of positives	15 (9 urines and 6 exhibits)	10 (7 bloods and 3exhibits)	3
Mean concentration µg/mL (range)	60 (10-260)	85 (40-200)	Femoral: 70, 130, 250 Urine: 500, 820, 5000
Cases received	1 700	1050	5 500
% of positive	0.9%	1.0%	0.05%

In all cases, the absorption was voluntary so at the moment no chemical submission case with this compound is reported in Québec. Generally the user takes that substance with numerous drugs like methamphetamine, cocaine, alcohol or cannabis. In GHB positive sexual assault cases, time between absorption and sampling ranges from 3 to 17 hours. In positive cases of driving under the influence of drugs, symptoms manifested by the driver are consistent with its major pharmacological effects; in 7 out of those 10 cases, other drugs were found in blood. In the 3 coroner's cases, GHB was not by itself directly related to the cause of death.

Conclusion: Our results show that GHB/GBL use in Québec is not as extended as reported by media. Based on those results corroborated by international studies, sedation and "black out" in the context of sexual assaults is generally due to consummation of alcohol or other xenobiotics. This presentation shows clearly that subjects usually take this drug with other substances in a recreational setting.

Keywords: GHB, chemical submission, driving under the influence of drugs

P58. Once upon a time, there was a little girl who suffered from insomnia… About a case of chemical submission discovered during a spell in hospital

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Introduction: The case of an 8-year-old girl without medical history brought to the emergency department of the Abbeville hospital is reported below. The fire brigade was called at 01:00 PM by the child mother because she did not succeed in waking up her girl. When she came at 03:30 PM at the emergency department, the girl had difficulties walking; she was euphoric and had auditory hallucinations. A blood sample was taken and a toxicological screening asked.

Methods: A routine screening for barbiturates, benzodiazepines, tricyclic antidepressants, ethanol and paracetamol was performed with an AxSym™ analyser. Alcohol level was assessed by GC-FID. Subsequently a screening by HPLC-DAD (Waters™, Alliance) and GC-MS (Shimatzu™) after solid/liquid extraction was done. Benzodiazepines with their metabolites (n=27) and sedative drugs (n=18) known to be used for chemical submission were tested for detection and assays by LC-MS/MS.

Results: The routine blood screening for the different psychotropic drugs and paracetamol turned out to be negative. The blood alcohol level was 0.3g/L. The screening was positive for valproic acid (140µg/L). As the child was not known for undergoing a treatment, the paediatric department was contacted. The girl reported having difficulties to fall asleep the previous night. She said that her stepfather gave her a "white tablet". Despite this, she woke up later that night. So she said that he then gave her 3 "pink pills" and 3 "white pills" with a nasty tasting drink.

The chromatographic analysis (LC-MS/MS) of a second blood sample taken 41 hours after these alleged events revealed nordiazepam (5ng/mL) and alprazolam (3.5 ng/mL). The chromatographic analysis of a 24 hour urine sample showed nordiazepam (3ng/mL) and its metabolite oxazepam (23ng/mL), alprazolam (11ng/mL) and its metabolite hydroxyl-alprazolam (125ng/mL). Symptoms presented by the child could fit the clinical signs described with a benzodiazepine administration (falling asleep and paradoxical syndrome).

Conclusion: Faced with a routine toxic screening requested by an emergency department, it seems necessary in case of a particular clinical situation to extend the analysis to an exhaustive toxicological screening. In the clinical case previously described, valproic acid and ethanol in the blood sample allowed to give the alert. Moreover this case confirms that chemical submission is not only a legal issue. Hospitals should also be aware of this problem.

Keywords: chemical submission, insomnia

P59. Screening for psycho-active drugs in case drug facilitated crimes: a case involving LSD

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Introduction: We present here a case of drug facilitated sexual assault involving LSD (lysergic acid diethylamide). Among the drugs that can be used for drug facilitated crimes, LSD, has been seldom observed [Pépin G. et al. Ann Toxicol Anal. 2002; 14: 395-406]. LSD, derived from lysergic acid which occurs naturally in the ergot of rye, is a potent hallucinogenic which is active at low doses.

Case report: A 23 years old man (addicted to cocaine and ecstasy), went with his friends in a disco. He drank half a glass before dancing and then finished his glass. 15 minutes after finishing his glass, he went to the toilet and suddenly had a memory loss about what happened before. When he regained consciousness, he was alone, very tired and diaphoretic. On his way home, he had disorders of the visual system. He was sent to a forensic institute for both clinical examination and toxicologic specimen sampling. He claimed to have consumed "speed" that day. Blood and urine samples were collected several hours after this.

Material and method: After liquid-liquid extraction, serum and urine samples (1 mL) in presence of two internals standards were analysed on a Acquity UPLC-TQD system (Waters). Separation was performed on a ACQUITY UPLC™ HSS C18, 1.8 µm (2.1 x 150 mm) column using a gradient of 5 mM formate buffer and acetonitrile with a runtime of 15 minutes. Detection was achieved by tandem mass spectrometry Acquity TQ Dectetor (Waters) operating in multiple reaction monitoring (MRM) mode. A screening of 136 compounds which can be involving in case of drug facilitated crimes was effected, such as benzodiazepines (flunitrazepam), hypnotics (zolpidem), sedatives (neuroleptics), drug of abuse, such as ecstasy or LSD. Two transitions were monitored for each compound.

Results: After UPLC-MS/MS screening, several drugs such as amphetamines, cocaine and LSD were identified in both serum and urine. The results are presented in following table:

	Serum (ng/mL)	Urine (ng/mL)
Cocaine	1	14,5
Benzoylecgonine	38	6 769
Ecgonine methyl ester	0.5	295
MDMA	20	12 544
MDA	3.5	495
Amphetamine	63	24 139
LSD	0.1	0.42
2 oxo 3 OH LSD	ND*	Presence

*Not Detected

Conclusion: MS/MS detection allows to the identification and confirmation of LSD in serum and urine at low concentrations. The performance of Acquity UPLC TQD allows a quick screening with high sensibility and sensitivity of many compounds involved in case of drug facilitated crimes.

Keywords: lysergic acid diethylamide, drug facilitated sexual assault, UPLC-MS/MS

P60. Development and evaluation of simple detection kit of organophosphates in urine for forensic and clinical cases

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Introduction: Organophosphates are widely used for pesticides. Although in normal circumstances they are non-toxic for humans, many people die through suicidal ingestion of organophosphates every year. An accurate, simple and handy method for detection of organophosphates in biological fluids is required in critical care practice. In this study, simple detection method of organophosphates in urine was developed without an expensive equipment. The detection kit has commercialized by using this mechanism in cooperation with Kanto Kagaku Co., Inc. (Tokyo, JAPAN). This kit was then applied to forensic and clinical cases in order to diagnose organophosphate poisonings and the results were compared with those of the GC-MS method.

Method: Urine (1.0 mL) was put into a detection tube by pre-coated with 4-(4-nitrobenzyl)pyridine. The tube was mixed by vortex-mixer for one minute and heated at 100 C for 20 min. After cooling to room temperature, 3 drops of tetraethylenepentamine was added to the tube and mixed by vortex-mixer for one minute. An extraction solvent was added to the tube in order to transfer organophosphate complex to an organic layer and easily diagnose by necked eye.

Results and discussion: The repeatability for data analysis of 27 organophosphates at the concentration of 50ug/mL are examined. The repeatability ranged from 0.30 to 4.2%. The detection limits of organophosphorus pesticides were 0.10 to 10 ug/mL in urine. Comparing with a commercial screening kit using a cholinesterase assay, the proposed method gave higher sensitivity and selectivity. The kit was applied to 12 poisoning cases who ingested organophosphates or other agricultural chemicals. In 4 cases, original organophosphates could not be detected in urine. We focused the fenitrothion poisoning, the compound was searched to cress-react with this kit. As a result of careful examination with GC-MS, metabolites (aminofenitrothion and S-methyl fenitrothion) that side chains were changed from nitro to amino or from methoxy to thiomethyl were identified in the urine. It has been cleared that the kit reacts to not only the parent organophosphates but also the metabolites.

Keywords: organophosphates, detection kit

P61. Extraction and derivatization of amphetamines and methylenedioxyamphetamines in urine using monolithic silica hold in a spin column and GC-MS analysis

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Introduction: As a new tool for sample preparation of drugs in biological materials, monolithic silica was packed in a spin column. The handlings such as sample loading, washing, elution of target drugs, were exhibited by a centrifugation of the spin column. In addition, many samples can be processed at the same time. This method has many advantages; easy operation, low volume of extraction solvent, and without evaporation. In this

study, the characteristics of the spin column hold by C_{18}-bonded monolithic silica were compared to those of a solid phase extraction cartridge. The pre-concentration efficiency of the spin column was excellent compared with the conventional solid phase extraction.

Method: Urine (0.5 mL), buffer (pH 13, 0.4 mL) and methamphetamine-d_5 (IS) were put into the pre-activated spin column and the column was centrifuged at 5,000 rpm for 3 min to load the sample solution. The column was then washed with the buffer by a centrifugation. The analytes adsorbed in the column were derivatized by adding 0.1 mL of ethyl acetate containing propylchloroformate to the column. After 5 min to finish a derivatization, the derivatized analytes were eluted by a centrifugation. The 1 μL-volume of the eluate was introduced to GC-MS.

Results and discussion: There was linearity from 0.01 to 5.0 μg/mL for methamphetamine and MDMA, and 0.01 to 1.0 μg/mL for amphetamine and MDA. The correlation coefficients of the calibration curves were more than 0.995. The coefficients of intra-day and inter-day variation at 0.1 and 1.0 μg/mL of amphetamines and methylenedioxyamphetamines in urine were 1.4 and 12.3%. The proposed method was applied to forensic and clinical poisoning cases. This spin column has a potential as a new tool for the routine analysis of drugs in biological materials.

Keywords: monolithic silica, derivatization, amphetamines

P62. Cholinesterase levels, symptoms and hematological parameters in flower growers exposed to organophosphate insecticides and carbamates in Montecillos, Cochabamba, Bolivia

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Introduction: In Bolivia one observes prolonged exposure to organophosphate insecticides and carbamates, pesticides of recognized danger, a fact that is reflected in the overdoses after applications of these products in the diverse cultures as is the case of the cultures of flowers, where the handling of these toxics is done without any type of precaution and control. Starting from this, the present investigation aimed at determining the levels of plasma cholinesterase and the concentrations of hematological parameters in blood.

Material and methods: The investigation covered 49 flower growers of both sexes (12 women and 37 men), occupationally exposed, of the community of Montecillos of the Canton Tiquipaya of the province of Quillacollo. The quantitative determination of the cholinesterase activity in blood (plasma) was performed with spectrophotometry (Wiener Lab.R) at 405 nm, using S-butyrylthiocholine Iodide as a substrate, at a temperature of 37 ºC. The reference range was 4,970 - 13,977 U/L. The determination of the hematological indicators (red and white blood cells, hemoglobin, hematocrit, and white blood cell differentiation) was performed by conventional methods.

Results: The obtained data, corresponding to the present situation, allowed to identify the levels of poisoning at the critical level (values less than 4,970), and at the intermediate level (values greater than 4,971), the relationship to the hematological indicators, the symptoms, the number of worked years, the sex and finally to the age groups.

Conclusion: The measured cholinesterase levels were in the critical range in 10.2% of the subjects and in the intermediate range in 89.8%.

Keywords: cholinesterases, organophosphate insecticides, carbamates, s-butyrylthiocholine iodide

P63. ACTP-Ester distribution and microsomal P450 analysis in goat

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Introduction: ACTP-Ester (Triclopyr butyl) is one of the important formulations of the herbicide triclopyr – an active ingredient of Garlon herbicide, used extensively in the agricultural field to control broad leaf weeds and woody plants. In order to study the distribution of ACTP-Ester and its two potential metabolites - triclopyr acid (3,5,6-trichloro-2-pyridinyloxy acetic acid) (M_1) and trichloro-pyridinol (3, 5, 6-trichloro-2- pyridinol) (M_2) from different substrates, the compound ACTP-Ester was administered orally to Black Bengal Goats (*Capra capra*). To evaluate possible metabolic pathway of ACTP-Ester the cytochrome P450 analysis was also considered.

Methods: ACTP-Ester was given at the dose of 396 mg/kg to each experimental goat. The control goats however, were treated with same amount of carboxymethyl cellulose (CMC). The gastro-intestinal (GI) tract contents of the animals sacrificed on 4,5,6 and 7 days post administration (pd) was recovered and quantified through standard protocol method of HPLC analysis. The urine and faeces samples however, were collected from each respective hr (i.e. at 24 hr till 168 hr. pd).

Results: The concentration of ACTP-Ester in rumen content was 17.69±1.07,15.16±1.97, 14.91±1.89, and 11.97±0.82 ppm on 4, 5, 6 and 7 days (pd) respectively. On the other hand, concentration of ACTP-Ester in small and large intestinal contents were 19.94±2.12 & 26.16±3.03, 17.14±1.09 & 12.13±1.02, 15.91±1.95 & 10.93±0.89, 8.87±0.86 & 5.39±0.53 ppm. The total amount of M_1 in GI tract content of goats sacrificed on those days (pd) were 19.06, 14.63, 16.11 and 21.58 mg respectively, whereas for M_2 the values were 1.66, 3.23, 4.48 and 6.99 mg respectively. The total amount of parent compound excreted as ACTP-Ester through faeces in goat sacrificed on those days were 158.88, 154.06, 177.68 and 184.68 respectively. The total quantity of M_1, however, recovered from faeces in such cases was 24.90, 23.42, 29.04 and 33.73 mg respectively and for M_2 it was 16.55, 16.41, 17.65 and 21.73 mg respectively. ACTP-Ester recovered from urine of goats was 74.78, 83.28, 71.47 and 84.86 mg respectively. The total quantity of M_1 recovered from urine in such cases was 1012.98, 1006.43 1088.72 and 1264.04 mg respectively and for M_2 it was 317.41, 346.42, 363.12 and 414.15 mg respectively. Maximum residual concentration of ACTP-Ester was recovered from bile (71.61±6.64 ppm) followed by lung (59.32±4.25 ppm), liver (50.11±3.61 ppm), heart (44.62±3.79), spleen (42.77±3.65 ppm) and brain (36.10±3.92 ppm). The presence of high amount of ACTP-Ester in those organs including brain showed gross cellular alterations due to the treatment. The concentration of M_1 increased in all tissues except brain, whereas maximum concentration for M_2 was detected in skin. ACTP-Ester treated goats did not vary in cytochrome P450 content from the control value.

Conclusions: Evidence clearly indicated that ACTP-Ester had moderate affinity to accumulate in tissues after oral dosing. Major excretory pathway of ACTP-Ester was directed through faeces while its metabolites M_1 and M_2 were predominantly excreted through urine.

Keywords: distribution, ACTP-Ester, goat

P64. Multi-method for analysis of 58 drugs of abuse compounds in full blood by aceton precipitation and LC-MS/MS analysis

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A method will be presented which is based on a combination of acetone precipitation of full blood samples and LC-MS/MS analysis. The uncomfortable combination of acetone and reversed phase analysis could be solved by using a non-conventional configuration of the UPLC high pressure pump (figure 1). The acetone extracts were injected prior to the mixing tee

into the strong mobile phase and diluted with water after the mixing tee to the initial mobile phase conditions. This resulted in a "weaker" solvent reaching the UPLC column and thus sharper peaks.

Aceton precipitation was favored over more HPLC compatible solvents because it produces clearer extracts. The resulting method was simple, fast and less labour intensive than previously used methods. After development the method was thoroughly validated according to ISO 170025 guidelines and in use for routine analysis since august 2007.

Figure 1. Schematic configuration of the high pressure pump.

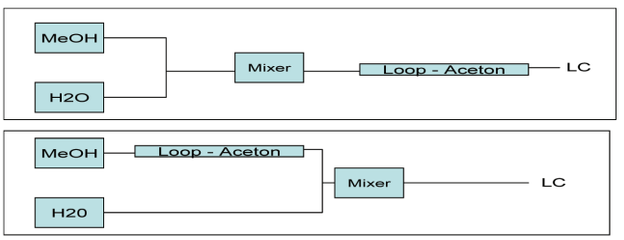

Table I. List of drugs of abuse compounds.

Compound	Compound	Compound	Compound
methylecgonine	Codeine	OH-ethyl-flurazepam	Midazolam
benzoylecgonine	MDMA	Alprazolam	9-COOH-THC
7-aminonitrazepam	nitrazepam	Temazepam	Citalopram
aminoclonazepam	clonazepam	Zolpidem	Fluvoxamine
Morfine	Zopiclon	desalk.flurazepam	Flurazepam
7acetamidonitrazepam	metamfetamine	Triazolam	Paroxetine
aminoflunitrazepam	desm.clobazam	Chloordiazepoxide	Fluoxetine
acetamidoclonazepam	lorazepam	1-hydroxymidazolam	Propoxyfeen
MDA	flunitrazepam	Lormetazepam	Methadon
6-mam	MDEA	Brotizolam	11-OH-THC
amfetamine	1-hydroxytriazolam	desm.diazepam	Nortriptyline
desm.flunitrazepam	Clobazam	Cocaine	Amitriptyline
demoxepam	desm. chloordiazepoxide	Diazepam	desm.tramadol
bromazepam	oxazepam	Tramadol	THC
	α-hydroxy-alprazolam	Lidocaine	

P65. Application of the Microgenics DRI® Ethyl Glucuronide assay to post-mortem cases and the investigation of drug-facilitated sexual assault (DFSA)

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Introduction: Ethyl Glucuronide (EtG) is a water soluble, stable, non-volatile, direct metabolite of ethanol. The elimination of ethanol by enzymatic conjugation with glucuronic acid represents approximately 0.5 to 1.5% of the total elimination. Whilst the detection period for alcohol is relatively short, EtG can be detected for up to 80 hours and peaks at approximately 4 hours after alcohol consumption. EtG offers several advantages over traditional markers of alcohol abuse such as gamma glutamyl transferase (GGT) mean corpuscular volume (MCV) and carbohydrate deficient transferrin (CDT). It is a direct, specific and sensitive marker of alcohol consumption, being present only if ethanol is consumed. It is not influenced by age, gender, medication or non-alcohol related disease and is not dependant on chronic alcohol consumption. Unlike urinary excretion of ethanol, EtG concentrations are highly influenced by water intake. Normalisation of EtG to creatinine is recommended. The Microgenics DRI® EtG assay is primarily targeted at alcohol abstinence, where zero tolerance policies are in existence. Such examples include liver transplant recipients and recovering alcoholics in withdrawal treatment programmes. We investigated its application in forensic settings, such as in post-mortem cases and in the investigation of drug facilitated sexual assault (DFSA).

Methods: EtG was measured in urine samples obtained from 20 DFSA cases and 8 randomly selected post-mortem cases, using the Microgenics DRI® EtG Enzyme Immunoassay on the Olympus AU400 platform. Assays were semi-quantitative (0, 100 (LLOQ), 500, 1000, 2000 (ULOQ) ng/mL) with 4 QC levels employed (375, 625, 750, 1250ng/mL). Cut offs of 500 ng/mL or 1000 ng/mL are typically recommended. Samples above the top calibrator for EtG were diluted using saline. EtG results were corrected for the effect of internal dilution using creatinine, measured using the Jaffe reaction on the Siemens Advia 2400 and were compared to ethanol concentrations measured by head Space GC-FID on the Shimadzu GC 2014.

Results: Numerous examples were identified in which EtG was present, indicating recent alcohol consumption, despite low or absent urinary ethanol. In isolation, alcohol concentrations in such post-mortem cases may be incorrectly attributed to fermentation. In one post mortem case high alcohol (297 mg/dL) with absent EtG was suggestive of bacterial glucuronidase degradation of EtG. A high EtG:creatinine (6463 ng/mmol) was detected in a late report (30hrs post incident) of sexual assault, with absent ethanol. This result may help to strengthen a case in which a defence of 'consent' has been made in the presence of demonstrable impairment.

Conclusion: EtG is a direct and more specific and sensitive indicator of ethanol consumption than traditional markers, such as GGT, MCV and CDT. The Microgenics DRI® EtG assay has application in not only clinical, but also forensic settings including Coroners' and DFSA cases.

Keywords: ethyl glucuronide, post-mortem, drug-facilitated sexual assault

P66. Relationship of methahemoglobin, ca[illegible]xyhemoglobin and hydrogen cyanide in victims of polyu[illegible]foam fire

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In a previous work in order to evaluate th[illegible]tion of carbon monoxide (CO) and hydrogen cyanide (HCN) as [illegible]t in fire causalities to the diagnosis of cause of death, a lethal [illegible] CO and HCN was defined (LI_{CO} and LI_{HCN}).

In the present work carboxyh[illegible] (COHb), hemoglobin (Hb), methaemoglobin (MeHb) and h[illegible]yanide (HCN) were quantified in victim's blood to elucidate th[illegible] the death. We analyzed data from blood samples of forensic a[illegible]ses in a tragic polyurethane mattress fire that provoked the deat[illegible]dividuals in 2005. The cadaveric blood samples were gently dr[illegible]uncturing femoral vein and analyzed by CO-oxymeter systems[illegible]lcohol (ethanol), cyanide and drugs were examined by gas ch[illegible]phy. Saturation of COHb ranged from 10% to 43%, Hb betwe[illegible]%, MetHb beetween 0.10 to 35.7% and HCN between 0.24 to 1[illegible]ese latter values were higher than the lethal levels reported in lite[illegible]er toxic components routinely measured (ethanol, methanol, ald[illegible]d other volatile compounds) gave negative results on the 32 case[illegible] drugs of abuse nor psychotropics were detected. The results in[illegible]at death in the 32 fire victims was probably caused by HCN, g[illegible]during the extensive polyurethane decomposition provoked by a r[illegible]ase of temperature. For other way, statistical analysis showed that[illegible] and %MetHb in blood concentration were not independent va[illegible]th χ^2=11.12 (theoretic χ^2=4.09, degrees of freedom=12, α=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 exponential decay r

$$\left[\frac{COHb}{MetHb}\right] = a^* \exp(-b^* MetHb)$$ where constants. With a non-linear regression model, the value was estimated through the representation of [CO-Hb/MetHb] on of MetHb. A good fitting with the experimental data was sing the proposed equation. The values of a=107.28±4.79, 06 and determination coefficients r^2=0.958. This is the fir at calculated the relationship between COHb and MetHb in d samples from polyuretane fires.

Keywords: carbo , hydrogen cyanide, methaemoglobin, fire deaths, CO-oxymeter

P67. Determination of cobalt in urine to discriminate its excessive administration using electrospray ionization tandem mass spectrometry

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Introduction: Cobalt (Co) is an essential element, and $CoCl_2$ is known to enhance erythropoiesis and angiogenesis. There is an emerging evidence that Co is taken as a supplement by athletes to improve anaerobic performance, resulting in increased Co concentrations occasionally observed in serum and urine. Excessive administration of Co, however, impairs thyroid activity and myocardial function, and promotes carcinogenesis. The limit of detection (LOD) of Co in the analysis should be lower than the concentrations in serum and urine of normal persons, ca. 0.3 mg/L, to discriminate the persons abusing Co. However, LOD of Co in the analysis based on conventional inductively coupled plasma (ICP) mass spectrometry (MS) was unsuitable; the LOD was 0.7 mg/L and the interferences due to polyatomic isobars corresponded to such a high level as 0.4 mg/L in serum. To obtain much better sensitivity using MS, electrospray ionization (ESI) tandem mass spectrometry (MS-MS) has been used in the present method.

Methods: Co was assayed as follows. To 10 mL of urine or standard solution in a polypropylene tube, 1 mL-aliquot of 1 M diethyldithiocarbamate (DDC) was added and mixed for 30 s with a vortex mixer. Then, 1 mL of 2 M citric acid and 10 mL of isoamyl alcohol (IAA) were added and mixed for 30 s, and then centrifuged at 5000 g for 30 s. ESI-MS/MS was performed on a TSQ 7000 LC-quadrupole mass spectrometer (Thermo Quest) in the positive ion mode. One mL of the IAA layer containing Co-DDC complex was injected manually in direct mode. The characteristic spectrum appeared 30 s after sample injection, and a sample could be injected every 60 s. Methanol was used as a mobile phase at 200 mL/min and the capillary temperature was set at 280°C. The electrospray voltage was set at 4.5 kV, multiplier voltage, 1.3 kV and collision voltage, 25 V, respectively. The quantification in ESI-MS/MS was performed by the integration of the peak area of the product ion $Co(C_4H_{10}NCS)_2^+$ at *m/z* 290.8±0.2 derived from the precursor ion $Co(DDC)_2^+$ at *m/z* 354.8±0.3.

Results: The concentrations of Co (y) in urine was linear to the concentrations of Co (x) spiked at 0.1-100 mg/L, y = 0.9978 x + 0.0867, with the correlation coefficient of 0.9991. Precision and accuracy were assessed for urine spiked at 0.1, 1, 10 and 100 mg/L and the coefficient of variation was less than 17.8%, and accuracy was between 81.1 to 121.8% for both intra-day and inter-day variations. The recoveries of Co were within 100±8% in three standard reference materials such as low level urine 2670a, high level urine 2670a and water 1643e obtained from NIST, Gaithersburg, USA.

Conclusion: Present method has enabled a sensitive determination of Co with LOD of 0.05 mg/L on ESI-MS/MS using 10 mL of urine within 10 min.

Keywords: cobalt, electrospray ionization, tandem mass spectrometry

P68. Analytical studies on benztropine and dextromethorphan

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Introdution: benztropine mesylate (BNZ), (1R, 3r, 5S)-3-Benzhydryloxytropane methanesulphonate is a tertiary amine antimuscarinic with action and uses similar to benzhexol, it also has antihistaminic properties used for the symptomatic treatment of parkinsonism. Dextromethorphan hydrobromide (DXT), [(+)-3-methoxy-17-methyl-9_, 13_, 14_-morphinan hydrobromide monohydrate is a cough suppressant, used for the relief of nonproductive cough.

A simple, accurate and highly sensitive spectrophotometric method is proposed for the rapid determination of benztropine (BNZ) and dextromethorphan (DXT) using alizarin red (Alz), picric acid (Pic) and phenolsulfonphthalein (PSP).

Method: The method depends upon extraction of the formed ion-associates into chloroform that exhibit absorption maxima at 429, 411 and 406nm in case of BNZ with Alz, Pic and PSP, respectively and at 425, 409 and 403 nm in case of DXT with Alz, Pic and PSP, respectively. The calibration curves resulting from the measurements of absorbance-concentration relations (at the optimum reaction conditions) of the extracted ion-associates are linear over the concentration range 4.50-90.78, 4.50-70.60 and 5.04-50.44 µg/mL for BNZ and 2.50-40.00, 5.00-40.00 and 1.50-16.00 µg/mL for DXT ion-associates with Alz, Pic and PSP, respectively.

Results: The stoichiometric ratio of the ion-associates (molar ratio method and contiuous variation method) and the effect of pH, temperature, time stability, influence of foreign ions, sequence of mixing, effect of shaking time and solvent power of extraction were were optimized to achieve high sensitivity, stability, low blank reading and reproducible results. The molar absorptivity and Sandell sensitivity of the reaction products were calculated.

Conclusion: Statistical treatment of the results reflects that the procedure is precise, accurate and easily applied for the determination of the DXT and BNZ in pure form and in their pharmaceutical preparations.

Keywords: benztropine mesylate, dextromethorphan HBr, alizarin red

P69. Simple and simultaneous detection of methamphetamine and dimethyl sulfone in crystalline methamphetamine seizures by fast gas chromatography

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Introduction: Methamphetamine (MA) is an addictive drug that stimulates the central nervous system. The drug illegally circulated in Japan is generally in the form of white crystals, so called "ice", and their purity is generally very high. However, in recent years, we have encountered MA-hydrochloride (HCl) seizures mixed with dimethyl sulfone (DMS) as a common adulterant agent. In this study, we present a simple method for simultaneous quantitative analysis of MA and DMS in crystalline MA seizures by fast gas chromatography (GC) with a narrow-bore (0.1 mm i.d.) capillary column.

Methods: A hundred milligrams of ground samples (seized MA crystals or powder) were put into a 50-mL measuring flask, and distilled water was poured to prepare 2 mg/mL aqueous solutions. A 0.5-mL volume of the solution was added to 1 mL of 80% potassium carbonate solution and extracted with 1 mL of dichloromethane/2-propanol (3:1) containing diphenylmethane as internal standard. After centrifugation, 1 µL of the extracts was subjected to GC analysis with a flame ionization detector on a DB-17 capillary column (0.1 mm i.d. × 5 m, 0.1 µm film thickness). The oven temperature was programmed from 100° to 175°X at 50°X/min. Helium was used as carrier gas at a constant column flow-rate of 0.5 mL/min.

Results: The use of a narrow-bore column gave fast and complete separation of 3 compounds within 1.3 min. One cycle of the temperature-programmed

analysis required only less than 3 min, realizing high-throughput detection. The calibration curves for both MA and DMS were linear over the range investigated (0.01–2.4 mg/mL, which are equivalent to 0.5%–120% in powdered samples). The lower limits of detection were estimated to be at 0.001 mg/mL for MA and 0.003 mg/mL for DMS (S/N≥3). The percent deviation of the intra- and inter-day accuracies was within 7% for the quality control samples including that of the lower limit of quantification (0.01 mg/mL). The intra- and inter-day assay precisions (CV) were also within 3.4%. The method was applied to the analysis of actual crystalline MA-HCl seizures. Out of 127 samples examined, 41 (32.3%) contained DMS, the contents of which were mostly less than 50%, though 6 samples contained more than 70% of DMS.

Conclusion: In this study, we have presented a detailed procedure for very simple and simultaneous analysis of MA and DMS in seized MA samples. This method seems very useful for preliminary analysis of MA and DMS in many samples, because of its simplicity and rapid GC detection. It seems applicable also to biological samples, though further experiments are needed to prove it.

P70. Experiences with the DrugWipe® 5+ Saliva rapid test for drugs of abuse at the roadside

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Introduction: The DrugWipe® 5+ rapid test was evaluated with the Zug and Zurich State Polices during a 7 months period in 2006/2007. The advantages of the DrugWipe® 5+ over the old DrugWipe® 5 are an enhanced handling while testing at the roadside and an improved sensitivity for different compounds. Saliva samples were collected from drivers suspected to be under the influence of drugs of abuse. The saliva specimens were tested by the police directly at the roadside using the DrugWipe® 5+ rapid test. In 59 cases of this study the police was able to receive a saliva sample, got clear results from the saliva test, ordered a medical examination of the driver, and a blood and urine sample could successfully be taken. No extra amount of saliva was collected. We compared the results of the DrugWipe® 5+ rapid test with the GC-MS findings in whole blood.

Method: The cut-off values of the DrugWipe® 5+ in saliva (DW) compared with our positive decision limits in whole blood (PDL) and the LOQ of our GC-MS methods in whole blood are as follows: Free morphine (DW 10 / PDL 15 / LOQ 10 ng/mL), free codeine (10/15/10), cocaine (15/15/10), benzoylecgonine (30/15/10), Δ^9-THC (30/1.5/1.5), 11-Nor-Δ^9-THC-COOH (2.0/1.5/1.5), d-amphetamine (50/15/10), d-methamphetamine (25/15/10), MDMA (25/15/10), and MDEA (65/15/10). All negative saliva test results were also checked in urine by an immunoassay test.

Results: The DrugWipe® 5+ rapid test results (n=59) were positive/false positive and negative/false negative for opiates (5/3 and 54/0), for cocaine (26/4 and 33/0), for cannabis (30/6 and 29/8), and for amphetamines (10/4 and 49/0). In the blood samples of the 59 subjects we found THC (28 times), MDMA (4), cocaine (19), ethanol (25), morphine (2), amphetamine (4), methadone (3), and benzodiazepines (1). 52 drivers out of the 59 subjects were considered to have driven while impaired, thereof 15 only by alcohol.

Conclusions: The police looked favourably upon the handling of the new DrugWipe® 5+ rapid test. For cocaine the test showed satisfactory results. Due to the small number of positive cases for opiates and amphetamines no conclusions could be drawn. And for cannabis the test was not satisfactory since 28% of the negative test results were false negative. For all analytes there were false positive, but only for cannabis false negative results.

Keywords: saliva; whole blood; rapid drug testing; roadside; driving

P71. Methamphetamine prevalence among arrestees in the UK

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Introduction: Methamphetamine is a sympathomimetic drug, which is a potent central nervous system stimulant. It is widely abused in the United States, Australia, the Far East and a number of European countries. It can be produced from widely available precursor chemicals in home laboratories. Previously published prevalence data[1] would suggest methamphetamine prevalence is very low. Anecdotally, its detection in analytical toxicology laboratories in the UK is rare. A small increase in the detection of home laboratories by UK police has been seen recently, however little is known about the prevalence of methamphetamine use across the UK. This study has been commissioned by the UK Home Office to determine the prevalence of methamphetamine amongst arrestees within the Drug Intervention Programme (DIP).

Methods: Samples were collected as part of the DIP from individuals arrested for acquisitive crime and tested on-site for cocaine and opiates using the Cozart Rapiscan. Arrestees within the DIP scheme were recruited for the study on a voluntary basis. 1199 samples were collected from 10 different police stations in geographical locations across the UK. The samples were screened for methamphetamine using the Cozart Spinlab DoA Liquid EIA kit for methamphetamine using the iLab 650 clinical chemistry analyser. Samples which screened positive for methamphetamine were analysed further using GC-MS to confirm the presence of methamphetamine and potential cross-reactants.

Results: 67 samples screened positive above the 45 ng/mL cut-off. 1 was confirmed positive for methamphetamine. 39 contained high levels of amphetamine (in excess of 1000ng/mL). contained high levels of MDMA. 8 samples did not contain any of the potential cross-reactants tested for, whilst 6 samples had insufficient volume remaining from the DIP programme testing to carry out a confirmation analysis.

Conclusion: From the cohort tested in this study it can be seen that less than 0.1% were positive for methamphetamine. Whilst care must be taken in interpreting these data since the testing was done on a voluntary basis and the cohort represents a limited section of society, the results are consistent with previously reported UK data [Clarke et al., Presented at TIAFT 2007, Seattle] and anecdotal evidence from UK analytical toxicology laboratories.

P72. Cannabis findings in drivers suspected of drugged driving in Finland

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Introduction: Alcohol and drug determinations of suspected driving under influence of alcohol and/or drugs cases are carried out centrally, at the National Public Health Institute in Finland. Finland has introduced a 'zero tolerance law' (per se law) that forbids operating a motor vehicle whilst an illicit drug or metabolite is present in the body. Cannabis is one of the most common findings among drugged drivers in Finland. The active component of cannabis is Δ^9–tetrahydrocannabinol (THC), and in the body THC is metabolized to 11- hydroxytetrahydrocannabinol (THC-OH) and tetrahydrocannabinolacid (THC-COOH). Interest of this work was to figure out how much more information about drug driving ivolving cannabis can be get by adding tetrahydrocannabinol metabolites to the analysing method. The drugged driving samples positive for cannabis from the database of the National Public Health Institute in the time period 2.1.2006-31.12.2007 are included in this study. The combinations and concentrations of THC and metabolites are presented.

Methods: Samples were first precipitated with acetonitrile, the supernatant was extracted with butyl acetate (BuAc). After evaporation of the organic

layer the residue was derivatized with ACN–MTBSTFA (50:20). The samples were then analyzed by GC–MS (SIM mode). The analysis method has been accredited and the cut off values for the method are 0.001 mg/L for THC and THC-OH, 0.005 mg/L for THC-COOH.

Results: The cases of this study were drugged drivers who had THC/ THC-OH / THC-COOH in their whole blood sample. Altogether 8949 samples were analyzed in the time period and cannabis was found in 23.1% (2067) of samples.

Found concentrations	n	minimum mg/L	maximum mg/L	mean mg/L
THC	982	0.0010	0.2000	0.0040
THC-COOH	2035	0.0050	0.3049	0.0220
THC-OH	386	0.0010	0.0235	0.0029

Combinations	%	n		minimum-maximum		mean
THC, THC-OH, THC-COOH	18.3	279	0.001-0.060	0.001-0.0236	0.005-0.3049	0.0068; 0.0029; 0.0050
THC, THC-COOH	27.6	571	0.001-0.0072		0.005-0.1236	0.0019; 0.0022
THC, THC-OH	0.09	2	0.0034-0,0078	0.0011-0.0014		0.0056; 0.0130
THC	1.5	30	0.001- 0.2000			0.0095
THC-OH, THC-COOH	0.2	5		0.001-0.002	0.021-0.0360	0.0015; 0.0029
THC-COOH	52.2	1080			0.005-0.0894	0.0011

Conclusions: The zero tolerance is applied if Δ^9–tetrahydrocannabinol or 11- hydroxytetra-hydrocannabinol are found from blood samples. The presence of THC-OH indicates 5 extra cases, negative for THC, which are punishable under the zero tolerance law. In addition 52.2% of cases (1080) were positive for the THC-COOH alone. Although these cases are not prosecutable under the per se law they provide useful information about the use of cannabis in driving cases. It is also seen that if THC is found alone or with THC-OH the mean concentrations of samples are higher than in the cases where also THC-COOH is found. This indicates more resent use of cannabis.

Keywords: cannabinoids, blood samples, drugs and driving

P73. A preliminary study on synthetic routes of designer drugs of the amphetamine type

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Introduction: Recently, chemical characterization based on the impurities and by-products present as a result of the synthetic method has became the basic procedure of drugs profiling. Identification of the synthesis route enables selection of the "specific" markers. Two main routes are used in clandestine laboratories in Europe to prepare common amphetamine-type stimulants, Leuckart reaction and reductive amination. Synthesis recipes of hundreds designer drugs were published by Shulgin. The present study was aimed at assessing efficiencies of mentioned synthetic routes for preparation of designer drugs of the amphetamine type.

Methods: Experiments were performed on 2,5-dimethoxyphenethylamine (2C-H), 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxy-4-iodophenylethylamine (2C-I), 2,5-dimethoxyamphetamine (DMA), 2,4,5-trimethoxyamphetamine (TMA-2), 3,4,5-trimethoxy-amphetamine (TMA), 2,5-dimethoxy-4-bromoamphetamine (DOB), 4-metoxyamphetamine (PMA) and 4-methylthioamphetamine (4-MTA) prepared according to so-called nitrostyrene method. It is a two-step synthesis involving condensation of an appropriate aldehyde with nitromethane or nitroethane, followed by reduction of the formed nitrostyrene. The crude products were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) on a HP-5MS column (30m×0.25mm×0.25µm) in a gradient conditions. Samples were prepared by liquid-liquid extraction from carbonate buffer (pH 9) using ethyl acetate, followed by vortex mixing and centrifugation.

Results: While syntheses of amphetamine either by Leuckart reaction or by reductive amination of phenylacetone are effective, their application to the synthesis of other arylpropan-2-amines or arylethylamines seems to be questionable. Both protocols would require synthesis of appropriate arylacetones or arylacetaldehydes, which are unstable, causing reactions impossible or at least non-effective. Thus, nitrostyrene method proposed by Shulgin was tested in further studies. The authors checked the efficiencies of 2C-H synthesis using lithium aluminium hydride ($LiAlH_4$) and aluminium hydride (AlH_3) and they were comparable (about 80%). The substitution of a nucleophilic reductor ($LiAlH_4$) by a reductor of electrophilic properties (AlH_3) enabled synthesis of halogenated derivatives of amphetamine and phenethylamine according to the same protocol. The yields of the crude amines (isolated as their hydrochloride salts) were satisfactory, from 61% for 2C-B to 95% for TMA-2. The worked-out GC-MS procedure enabled qualitative and quantitative analysis of prepared designed drugs as well as assessing the impurity content.

Conclusions: The formation of nitrostyrenes followed by their reduction using aluminium hydride as proposed by the authors seems to be universal synthesis method of amphetamine and phenethylamine derivatives. The analysis of content of main compound and its impurities according to the worked out GC-MS procedure can be useful for determination of drug origin.

Acknowledgments: This work was supported by the project O N204 0060 33 of the Ministry of Science and Higher Education, Warsaw, Poland.

Keywords: designer drugs; amphetamines; synthesis

P74. Characterization of Shabu (methamphetamine) sold in Iloilo City

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Introduction: Knowledge of impurities and major precursors in methamphetamine is important since it can reveal information on the synthetic methods used to produce the drug. Over the years, United Nations Office on Drugs and Crime (UNODC) activities has focused on methamphetamine and its main precursor, ephedrine. South-East Asia, has been particularly affected by the clandestine manufacture of, trafficking in, and abuse of methamphetamine. For trafficking purposes, methamphetamine samples are often adulterated with ephedrine. The aim of this study is to characterize methamphetamine being sold in the market as well as to detect impurities such as starting materials and precursors in illegally prepared methamphetamine in Iloilo City, Philippines.

Methods: A transparent crystalline substance which was suspected to be methamphetamine was isolated and purified by the acid-base extraction. Impurities were extracted with chloroform and the sample was analyzed to contain 5.06%±0.006 methamphetamine. Major peaks were selected for comparison and similarity and/or dissimilarity between street sample and purified sample using Fourier Transform Infra Red analysis (FT-IR).

Results: Results indicate the presence of methamphetamine (5.06%±0.006) in the sample with dl-ephedrine as the major adulterant (94.94%) as shown by the FT-IR results. Furthermore, Gas Chromatography- Mass Spectroscopy (GC-MS) analysis is reported for the sample to contain methamphetamine as indicated by standard identifying peaks.

Conclusions: Analytical data of the shabu sample (color test, melting point, FT-IR, GC-MS) confirmed that the street sample contain methamphetamine.

The acid-base extraction method employed was effective in isolating methamphetamine from the street sample. The percentage methamphetamine in the street sample as determined using a gravimetric method is 5.06%±0.006. The possible major adulterant present is dl-ephedrine (94.94%) based on the melting point and FT-IR results. It is recommended that the adulterant should be studied further to verify the results using GC-MS. It is also recommended that the % methamphetamine in the street sample should be measured using GC-MS to validate the result of the gravimetric analysis.

Keywords: shabu, FT-IR, GC-MS

P75. Simultaneous detection of para-methoxymethamphetamine, para-methoxyamphetamine, 2,5-dimethoxy-4-ethylthiophenethylamine and 2,5-dimethoxy-4-(n)-propylthiophenethylamine in urine by gas chromatography–mass spectrometry

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Aims: A sensitive and specific analytical method for the quantification of synthetic designer drugs in urine was needed and developed.

Methods: urine sample added with self-synthesized internal standards of PMA-d3, 2C-T-2-d6, and 2C-T-7-d6, and 1 mL of 0.1 M phosphate buffer were extracted using Varian SPEC® DUA solid phase extraction column. The analytes were eluted with 1 mL dichloromethane containing 2% concentrated ammonia solution. Derivatization with acylation or silylation reagents were evaluated. Ethyl acetate was added to dissolve the derivatized drugs after drying by purging, then quantified with an Agilent 6890GC/5975MS system in EI mode using selective ion monitoring, quantification and qualification ions with low cross contribution were selected.

Results: The acylation products from heptafluorobutyric anhydride, pentafloropropionic anhydride and trifluoroacetic anhydride showed better electron impact mass spectra than the silylation products and acylation was used subsequently. Calibration concentration range was 50 to 1500 ng/mL and the coefficient of correlation (r^2) exceeded 0.992 for all of analytes. Slightly better sensitivity were shown for HFBA derivatization than TFAA and PFPA, LOD was 20 ng/mL for PMA, PMMA, 2C-T-7, and 40 ng/mL for 2C-T-2. LOQ is 40 ng/mL all four analytes. Using HFBA, the accuracy of intra-day and inter-day tests at 3 different concentrations ranged from 89.3% to 105.1%, and CVs were all within 7.2%. The method was applied to two real urine specimens, the concentrations of PMA and PMMA were 276 ng/mL, 831 ng/mL in one, and 455 ng/mL, 1,450 ng/mL in the other respectively.

Conclusion: The method has been validated and showed good sensitivity, specificity, accuracy, precision and suitable for real urine specimen analysis.

Keywords: PMMA, PMA, 2C-T-2, 2C-T-7, urine, GC-MS

P76. Reference substances for plant materials of toxicological interest

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Introduction: Plant products have been used as toxins since historic times. Toxic plant material can also appear, by accident or design, in herbal products offered for sale. In addition, as access to illegal drugs is strictly controlled, a trend has been noticed with websites and underground literature directing consumers seeking psychoactive effects to plants and plant products for their intoxicating potential and their ease of procurement. As a result, forensic science institutes continue to report the need for well characterised reference substances for materials of plant origin which have toxic, hallucinogenic, psychedelic, stimulating or relaxing effects. Some phytochemical reference materials are available from specialist suppliers; however these sources have not traditionally supplied forensic institutions and are not aware of their needs. Conversely, forensic institutions are not well informed about sources of phytochemical reference materials.

An initiative has been mounted to establish which phytochemical reference materials are of potential interest to forensic institutions and which are commercially available.

Methods: We consulted a small group of forensic toxicologists and drugs analysts to establish their "wish list" of plant reference materials. We then reviewed the phytochemical reference standards to identify which are commercially available.

Results: A listing of available phytochemical reference materials of relevence to forensic toxicologists is being developed. The current listing includes materials such as:

Systematic name	Common name	Examples of phytochemical(s) available
Aconitum spp	Monkshoods	Aconitine, bulleyaconotine, etc
Amanita muscaria	Fly agaric	Ibotenic acid, muscimol, muscarine, etc
Argyreia nervosa	Hawaiian baby woodrose	Ergine, isoergine, ergonovine
Aristolochia	European Birthwort	Aristolochic acids
Atropa belladonna	Deadly nightshade	Atropine, scopolamine, tropine, etc
Banisteriopsis caapi	Ayahuasca	Harmine, harmaline
Brugmansia aurea	Angel´s trumpet	Atropine, scopolamine, hyoscyamine, etc
Ipomoea violacea	Morning glory	Ergine, ergonovine
Mitragyna speciosa	Kratom	Mitragynine, 7-hydroxymitragynine
Piper methysticum	Kava kava	Kavain, dihydrokavain, etc
Psolocybe spp	Magic Mushrooms	Psylocin, psylocybin
Salvia divinorum	Salvia	Salvinorin A

Conclusion: A reference list of available phytochemical reference materials will be a useful tool for forensic toxicologists. A list of hard, or impossible, to obtain materials will also be of value to encourage producers of phytochemical reference materials to develop new materials of use to forensic toxicologists. We would therefore welcome recommendations from practising toxicologists of phytochemical reference standards which they would like to be able to obtain to support their work.

Keywords: plant materials, reference substances, forensic toxicology

P77. Determination of cotinine in perdicardial fluid and whole blood by liquid chromatography-tandem mass spectrometry

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Introduction: A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the determination of cotinine concentrations in pericardial fluid and whole blood. Cotinine is the main metabolite of nicotine and is used as an indicator of exposure to tobacco smoke. During autopsy, clean whole blood samples may be difficult to collect and pericardial fluid (the fluid that surrounds the heart) may be an alternative sample material.

Methods: Whole blood and pericardial fluid samples from 99 sudden infant deaths were obtained during autopsy between 1990 and 2004. To 0.5 mL whole blood, 50 µL cotinine-d_3 was added. The mixed blood sample was precipitated with 1.8 mL ice cold acetonitrile and placed in freezer (-20ºC) for 30 min. The supernatant was extracted by solid-phase extraction with a polymer-based mixed-mode column (Oasis MCX). Pericardial fluid (1 mL) was prepared by addition of 100 µL cotinine-d_3 prior to solid-phase extraction. Chromatographic separation of extracts was achieved using a Waters Atlantis dC18 (2.1x50 mm, 3.5 µm) column with a flow rate of 0.3 ml/min, with gradient elution. The analyses were performed on a Waters Alliance 2695 system in combination with a Waters Quattro Ultima Pt tandem-quadrupole mass spectrometer equipped with a Z-spray electrospray

interface. Positive ionization was performed in the MRM (multiple reaction monitoring) mode. Two transitions were monitored for the analyte, and one for the internal standard.

Results: The concentration range was 5-1000 nM for cotinine in both matrixes. The average recovery of the analyte ranged from 86-92% and the between-assay precisions ranged from 4 –6% RSD. The limit of quantification was found to be 2 nM. A strong correlation (R^2=0.97) was found between pericardial fluid and blood for cotinine.

Conclusion: The LC-MS/MS method proved to be robust and specific for the determination of cotinine in both blood and pericardial fluid. Pericardial fluid may be an alternative sample to blood for cotinine analysis in forensic autopsies as a strong correlation between the cotinine concentrations in the two matrixes was demonstrated.

Keywords: cotinine, pericardial fluid, blood, LC-MS/MS

P78. Determination of 4 benzodiazepines with direct injection of whole blood by a column switching technique

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Introduction: A simple, sensitive and reproducible method was developed for determination of four benzodiazepines (Clonazepam, Flunitrazepam, Triazolam and Diazepam) with direct injection of whole blood by column switching and UV detection.

Methods: Whole blood samples were diluted 1:1 with water and then directly injected onto an Onyx Monolithic C18 column for a preliminary clean-up and then separated with a C18 150 × 4.0 mm I.D. reversed-phase column at room temperature. The mobile phase of the monolithic column consisted of pH 2.5 phosphate-acetonitrile buffer (85:15 v/v) at flow rate of 2.5 ml/min. The mobile phase of the analytical column consisted of pH 2.5 phosphate-acetonitrile buffer (60:40, v/v) at flow rate of 1.0 ml/min. The detection was carried out at 240 nm. No internal standard was necessary.

Results: The method was linear over a concentration range of 0.1-10 µg/mL for Flunitrazepam and Triazolam. For Clonazepam and Diazepam, linearity was over the range 0.3-10 µg/mL. Quantification limits ranged from 0.1 to 0.3 µg/mL and detection limits from 0.05 to 0.1 µg/mL. Recovery ranged from 95% to 98%. Within-day and between-day coefficients of variation ranged from 2.1% to 6.2%.

Conclusion: The present method has been applied successfully to the determination of toxic concentrations of benzodiazepines in forensic toxicology.

Keywords: benzodiazepines, monolithic column, column switching

P79. First case report of recreational use of diphenylprolinol (diphenyl-2-pyrrolidinemethanol (D2PM)) confirmed by toxicological screening

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Introduction: A 21 year old male ingested 3 tablets of 'head candy' purchased in a local high street shop. Following ingestion, he felt mildly euphoric for several hours. As the effects were wearing off he had 2 'sniffs' of "poppers" (alkyl nitrite). He then became dizzy and developed chest discomfort and a feeling of heaviness in the right arm, precipitating his presentation to the Emergency Department (ED). On arrival, he was agitated with a heart rate of 126bpm and blood pressure of 213/109 mmHg. He was apyrexial and had unassisted oxygen saturations of 100%. His pupils were dilated but reactive to light; the remainder of his neurological examination was normal. His admission electrocardiogram (ECG) showed a sinus tachycardia, with normal QRS and QTc duration. Biochemical, haematological and blood gas investigations were normal on admission. He was treated with benzodiazepines for both his agitation and hypertension. His methaemoglobin concentration was measured and found to be normal (0.5%). He was admitted for overnight observation and treated with a total of 20mg of oral diazepam. His symptoms, agitation and hypertension settled with this management and he was discharged asymptomatic the following morning.

Methods: After the addition of internal standard (SKF525A and flurazepam), samples and calibrators (100µL) were extracted with methyl-*tert*-butyl-ether (MTBE) under alkaline conditions and recovered from the resulting organic phase into 0.1M phosphoric acid. The aqueous layer was made alkaline, re-extracted with MTBE and a 1.0µL aliquot of the supernatant analysed by GC-MS following splitless injection. This was performed using a Shimadzu GC-MS-QP2010 with an AOC-20i autosampler and HP-5MS (30m x 0.25mm, 0.5µm; 5%-Phenyl-methylpolysiloxane) column. Helium was used as the carrier gas at a flow rate of 1mL/min. The injector was maintained at 225°C and the detector at 200°C. The initial column temperature was set at 150°C and held for 4mins. It was then ramped by 30°C/min up to 290°C and held for 9.33mins, giving a total run time of 18mins. Positive Electron Impact Ionisation mode was used and data were collected using single ion monitoring (SIM). D2PM, SKF525A, glaucine and flurazepam were quantified monitoring their most abundant ion (*m/z*: 70, 86, 354 and 86 respectively). Their retention times were 9.43, 10.36, 15.52 and 14.21 minutes respectively.

Results: Routine toxicological screening of the blood and urine identified the presence of diphenylprolinol (diphenyl-2-pyrrolidinemethanol (D2PM)) and glaucine. Blood concentrations of D2PM and glaucine were estimated at 0.10mg/L and 0.17mg/L, respectively. No other drugs or alcohol were detected using a broad toxicology screen.

Conclusion: A more systematic approach to toxicological screening in patients with recreational drug toxicity is required, not only to identify novel or emerging drugs, but to provide a more systematic means of monitoring trends and providing evidence for legislative authorities.

Keywords: diphenyl-2-pyrrolidinemethanol, glaucine, head candy

P80. An ONLINE DAT® immunoassay for the detection of benzodiazepines and glucuronidated analogues in urine

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Introduction: A new assay is in development for the detection of benzodiazepines and their glucuronidated metabolites in urine on automated clinical analyzers*.

Method: The liquid, two reagent, ready to use homogeneous assay utilizes the KIMS technology (Kinetic Interaction of Microparticles in Solution), where drug conjugates are covalently linked to carboxy-modified polystyrene microparticles, and purified polyclonal antibody and a beta-glucuronidase enzyme are in solution. The assay utilizes cutoff concentrations of 100, 200 and 300 ng/mL for both semi-quantitative and qualitative methods, features an extended dynamic range compared to currently available assays and exhibits higher cross-reactivity to glucuronidated species compared to assays without the enzyme.

Results: Studies summarized herein evaluate the 100 ng/mL cutoff (comparable results obtained for the 200 and 300 ng/mL cutoff). When run in semi-quantitative mode on Roche/Hitachi 917 analyzers, control recovery at 50, 80, 100, 120 and 600 ng/mL showed a recovery of 50.8 (101.5%), 79.8 ng/mL (99.7%), 101.7 ng/mL (101.7%), 117.3 ng/mL (97.8%) and 610.8 ng/mL (101.8%) with intra-assay %CV precision values of 3.4%, 1.7%, 1.6%, 1.5% and 0.5%, respectively. The inter-assay precision of the same levels ranged from 2.2 to 7.4%. Lorazepam glucuronide, oxazepam glucuronide, and temazepam glucuronide show cross-reactivities of 38.5%,

42.67%, and 40.33% cross-reactivity with the enzyme compared to cross-reactivities of 1.34%, 0.87%, and 1.09% without the enzyme, respectively. An internal method comparison of 50 suspected positive samples analyzed against the CEDIA assay showed 11 samples that gave <100 ng/mL results with CEDIA but positive results with the ONLINE DAT assay. These 11 samples all showed the presence of benzodiazepines upon GC/MS confirmation. The remainder of the samples all showed agreement between the methods. GC/MS confirmed negative clinical samples give an average reading of 1.4 ng/mL.

Conclusion: The above studies demonstrate that this assay provides an accurate and precise method for screening urine for the detection of benzodiazepines and their glucuronidated analogues on automated systems.

*This assay is currently in development and has not been cleared or approved for use in the US by the FDA. ONLINE DAT and CEDIA are trademarks of Roche.

Keywords: benzodiazepine, immunoassay, glucuronidase

P81. Report from a proficiency testing program, Nordquant, 1989-2007: has quantification of drugs in blood improved?

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Introduction: The quality and reliability of toxicological analysis are of major importance in forensic cases (victims, suspects, autopsies). For this reason our laboratory started a proficiency testing program, Nordquant, in 1989. Blood batches were spiked with drugs in variable concentrations and sent to the participants twice annually. Participants knew which drugs to analyze for. This presentation gives results for 10 drugs that have been in the scheme the whole time: amitryptiline, amphetamine, codeine, diazepam, flunitrazepam, methadone, morphine, nitrazepam, propoxyphene and tetrahydrocannabinol (THC). The present study was undertaken to see whether the quality of the results have changed through this 18-year-period.

Method: Data were divided into three time periods, each including 12 rounds: 1989-1994(period 1), 1995-2000(period 2) and 2001-2007(period 3). For each of the 10 drugs median and mean values, standard deviations (SD) and coefficient of variance (CV) for each round were calucaled. The CVs of each period were aggregated, and the periods compared by one-way analysis of variance, using "Analyse-it for clinical chemistry" v.1.72 (2004, Analyse-it, Ltd, UK).

Results: The number of participating labs were 6 in 1989 and 14 in 2007. Most labs have increased the number of drugs in their repertoire; for example was THC analysed by two labs in 1989 and 8 labs in 2007. The mean theoretical (spiked) concentrations were not significantly different between periods for any of the drugs. Calculation of CVs for period 1, 2 and 3, respectively, showed statistical significant decreases for codeine (26,7%; 29,6%; 16,1%; p=0,021, period 2 vs. 3), morphine (31,6%; 28,1%; 18,7%; p=0,020, period 1 vs. 3) and diazepam (27,4%; 21,1%; 17,3; p=0,034, period 1 vs. 3). Non-significant decreases were observed for amitryptiline, amphetamine, methadone, nitrazepam, propoxyphene and THC. For flunitrazepam an extremely high CV of 96,3% for period 3 was observed, mainly due to one lab. The mean CVs for all drugs taken together were 38,2%, 31,5% and 31,4% for the three periods.

Conclusion: The NordQuant program shows that over all analytical precision has improved slightly from 1989 to 2007. Refined methods and better instruments are available now. Increased awareness of quality, including lab accreditation, may have contributed.

Keywords: Proficiency testing, Nordquant, quality control

P82. Determination of four nitrofuranes group antibiotics metabolites in honey by liquid chromatography-ion trap mass spectrometry

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Introduction: The inspection and controlling of banned nitrofurans (furazolidone, furaltadone, nitrofurantoin and nitrofurazone) in foodstuff is required due to the significant concerns about carcinogenicity and mutagenicity of their respective metabolites, 3-amino-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-1,3-oxazolidin (AMOZ), 1-aminohydantoin (AHD) and semicarbazide (SEM). The aim of the study was to develop a rapid, sensitive and selective method for simultaneous determination of 4 nitrofuran metabolites in honey using liquid chromatography with electrospray ionization ion trap mass spectrometry (LC-ESI-MS/MS) in positive mode.

Methods: On 2 g of honey, 0.125 M hydrochloric acid and the 50 mM NBA derivatization agent were added. The slurry was then incubated, after cooling at room temperature; sample neutralized by 1% ammonia solution and the final pH was adjusted to 6.5-7.0. The SPE cartridge conditioned with 1 mL of methanol followed by 1 mL of the water. Neutralized sample solution was loaded on the column. The cartridge was then washed with 3 mL of water than dried by sucking air through after washing step with vacuum. The derivatized nitrofuran metabolites were finally eluted with 3 mL of acetonitrile, evaporated to dryness. The residue was dissolved in 250 µL of methanol/water (30:70 v/v).

Results: The method was found to be selective for the four nitrofuran metabolites. The results for LOD, LOQ, accuracy, the precursor ions and fragment ions presented in the table below. The relative standard deviations between parallel extraction and each three replicated injections gave accuracy of the method. Relative standard deviation of repeatability ranges between 0.25-9.67% is satisfactory with regard to the performance required for analytical methods.

Substance	**MRM** quantification (m/z)	**MRM** confirmation (m/z)	**LOD** (µg/kg)	**LOQ** (µg/kg)	**Accuracy** RSD % (1.0 µg/kg)
AMOZ	335.0>291.0	335.0>262.0	0.18	0.55	4.75
AHD	249.0>134.0	249.0>178.0	0.80	2.50	1.64*
AOZ	236.0>134.0	236.0>121.0	0.16	0.48	0.42
SEM	209.0>166.0	209.0>192.0	0.35	1.08	0.96

Conclusion: After optimization of chromatographic conditions, the method was validated. Specificity was studied extendedly and any probable or questionable interference was not observed. Tandem mass spectrometry using MRM transitions with two time segments enable a selective and confirmatory detection at the sub parts-per-billion level, below the MRPL set at 1 µg/kg except 2-NBA-AHD. Described method can be used for quantitative and qualitative determination of metabolites of banned nitrofurans.

Keywords: nitrofuran, LC-MS/MS, validation

P83. Screening of drugs and drugs of abuse using gas chromatography-mass spectrometry; application to human urine analysis

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Aims: The object of the study was to develop a GC-MS method for screening and semiquantification of drugs and drugs of abuse in human urine. The separation power of GC as well as the selectivity of the detection of MS,

make GC-MS the technique of choice for systematic toxicological analysis. Matching both the retention time and full scan spectra of peak of unknown compound with a standard is proof of identification.

Methods: After liquid-liquid extraction of 2 mL of drug-free human urine (at pH 8-9 basic and neutral analytes were extracted with 4 mL of ethylacetate:1-chlorbutane:cylohexane (3:1:1 v/v/v), at pH 2 acidic analytes were extracted with 4 mL of ethylacetate:toluene (4:1 v/v)), the 20 analytes codeine, morphine, ephedrine, 3,4-methylenedioxymethamphetamine, tramadol, dothiepin, cocaine, mirtazapine, clomipramine, alprazolam, zolpidem, clozapine, amitriptyline, citalopram, diazepam, levomepromazine, bromazepam, phenobarbital, guaifenesin and internal standards trimipramine d_3 and hexobarbital were separated on HP-5ms 30 m x 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. Validation of the method included evaluation of recovery, linearity and repeatability. The application of the described assay was tested by analysis of real samples. After acid hydrolysis of one aliquot of urine another aliquot of native urine was added and the mixture was liquid-liquid extracted and GC-MS analyzed. The detection of compounds with active groups including free hydrogen atoms in the molecular structure were performed after silylation.

Results: The analytes were sufficiently separated and sensitively detected. The presence of the analytes was successfully screened for by mass chromatography with selected ions followed by library search. The calibration curves of the analytes were linear in concentration range 0.05-2.0 µg/mL with correlation coefficients exceeding 0.995. The limit of quantification for all analytes was 0.05 µg/mL. The repeatabilites expressed as relative standard deviations ranged from 1.5 to 10%. The extraction efficiencies were tested on concentration levels 0.1 and 1.0 µg/mL and estabilised in range 75 – 102%.

Conclusion: The presented full scan GC-MS method allowed screening as well as identification and semiquantification for 20 of the most commonly encountered drugs and can be successfully applied to analysis of samples from real clinical and forensic toxicology cases.

The study has been supported by the grant IGA MZ NR 9365-3/2007

Keywords: screening, gas chromatography-mass spectrometry, drugs

P84. A GC-MS method for the determination of cyclic antidepressants and their metabolites in urine with data comparing free and glucuronide bound drug concentrations

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Aims: The aim of this study was to develop a fast, sensitive, and selective GC/MS method for simultaneous determination of secondary and tertiary tricyclic antidepressants (TCAs) in urine and to study the extent of glucuronic conjugation of parent TCAs and their metabolites by applying enzymatic hydrolysis.

Methods: The samples (2 mL urine with maprotiline D_3 as internal standard) were hydrolyzed by adding 0.5 mL of a 12% β-glucuronidase (from E. coli) solution in 0.2 M phosphate buffer (pH. 7.0) and incubating at 52 °C for one hour. Target analytes were then isolated from alkalinized urine using heptane/dichloromethane/dichloroethane isopropyl alcohol (10:5:5:1). Two aliquots of each specimen were extracted, one with and the other without hydrolysis. The final dried extracts were derivatized with MSTFA/ammonium iodide/ethanethiol reagent (50 mg/25 mL/75 µL). Analysis was carried out using an Agilent GC/MS, capable of fast temperature programming, operating in EI/SIM mode. Separation was performed on a CP-SIL 5CB (10 m x 0.15 mm, film thickness 0.12 µm) column with hydrogen as the carrier gas.

Results: The method was fully validated, including linearity (50-20,000 ng/mL for amitriptyline, imipramine and desipramine; 50-10,000 ng/mL for nortriptyline and maprotiline; 50-2000 ng/mL for doxepin and desmethyldoxepin), inter day and intra day precision (<6%), limit of detection (50 ng/mL for doxepin and desmethyldoxepin; 25 ng/mL for all other analytes), limit of quantitation (100 ng/mL for doxepin and desmethyldoxepin; 50 ng/mL for all other analytes), specificity (no exogenous interference from other spiked drugs), matrix effect (no endogenous interference from urine matrix), different derivatizing schemes and stability of extracts after 48 hours at room temperature. The procedure was applied to 24 clinical specimens screened positive by preliminary TLC screen. Free amitriptyline and nortriptyline was detected in 12 samples, imipramine, desipramine and OH-desipramine metabolites in 2 samples, desipramine and OH-desipramine metabolites in 5 samples and doxepin and desmethyldoxepin was detected in 5 samples. Following hydrolysis, there was approximately 10 times increase in amitriptyline and doxepin and 2-4 times increase in imipramine concentrations. No significant increase in the concentration of nortriptyline, desipramine and desmethyldoxepin was noted following hydrolysis. The hydroxy metabolites were qualitatively detected and were also found to be glucuronide bound. There were no clinical specimens available containing maprotiline, therefore no comment can be made regarding conjugation of parent maprotiline in urine at this time.

Conclusions: Tertiary TCAs are extensively glucuronide bound in urine and enzymatic hydrolysis markedly increases their recoveries. In addition, the use of MSTFA/ammonium iodide/ethanethiol derivatizing reagent offers advantages over trifluoroacetylation procedures by selectively derivatizing the secondary TCAs while eliminating the decomposition of the tertiary TCAs. The use of fast GC with a narrow bore capillary column and hydrogen as a carrier gas, results in a fast, simple, sensitive and specific method for the quantitative determination of common TCAs along with the qualitative determination of their hydroxy metabolites, in human urine.

Keywords: tricyclic antidepressants, glucuronide conjugation, urine

P85. New chromatographic method for the isolation of Δ9-tetrahydrocannabinolic acid A (Δ9-THCA-A) from Cannabis sativa

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Introduction: More than 400 compounds have been identified in *cannabis sativa*, about 60 belonging to the class of cannabinoids. Δ9-Tetrahydro cannabinolic acid (Δ9-THCA-A) is the predominant compound of the cannabinoid fraction and the non-psychoactive biogenetic precursor of Δ9-Tetrahydrocannabinol (Δ9-THC). In fresh plant material about 90% of the available Δ9-THC is present as Δ9-THCA-A. When heated or smoked, Δ9-THCA-A is partially converted to Δ9-THC by decarboxylation. Several procedures have been published for the isolation of Δ9-THCA-A from hemp, but all of these use either expensive sorbent material or harmful organic solvents. The aim of the presented work was to establish a simple and cost-saving procedure for the isolation of Δ9-THCA-A from hemp without the use of toxic solvents such as dichloromethane/chloroform or toluene.

Methods: The pulverized plant material was extracted with ethanol at 4°C for 48 h. After filtration, the crude extract was treated with activated carbon, re-filtrated and evaporated to dryness yielding a brown amorphous residue. For purification, the residue was chromatographed on a conventional preparative column (40 mm x 400 mm) filled with 120 g silica 60. Δ9-THC was eluted using cyclohexane/ethyl acetate (20:1, v/v, plus 500 µL/1 L formic acid). For the elution of the more polar Δ9-THCA-A the composition of the eluent was changed to cyclohexane/ethyl acetate 20:2, v/v, plus 500 µL/1 L formic acid. The Δ9-THCA-A positive fractions were collected and carefully

evaporated to dryness. The yellow residue was reconstituted in HPLC solvent (aq. ammonium acetate (5 mM, pH 6.5)/methanol (25:75, v/v)). Aliqouts (250 µL) were separated by preparative HPLC (Agilent G1361A, 1200 preparative pump, Phenomenex Hydro-RP, 250 x 15 mm, 10 µm; aq. ammonium acetate (5 mM, pH 6.5)/methanol (25:75, v/v), 30 mL/min) using UV detection at 220 nm. The eluent fractions containing Δ9-THCA-A were collected, acidified with formic acid and re-extracted with *tert*-butyl methyl ether (TBME).

Results: Using a simple preparative column chromatography, Δ9-THC and other secondary plant constituents were for the most part removed. Δ9-THCA-A could then be separated from the remaining traces of Δ9-THC by preparative HPLC within a 10 minutes run and the re-extraction with TBME proved to be suitable for isolation of Δ9-THCA-A from the mobile phase.

Conclusions: The described procedure presents a simple, non-hazardous and – compared to patented procedure - cost-saving method for the isolation of Δ9-THCA-A from hemp. Conventional column chromatography followed by a standard preparative HPLC proved to be suitable for preparation of larger amounts of Δ9-THCA-A which are needed for kinetic studies of this novel cannabis consume marker.

Keywords: Δ9-Tetrahydrocannabinolic acid A, *Cannabis sativa*

P86. Comparison of MRM vs. full scan MS/MS for LC-MS/MS drug confirmation

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Introduction: The use of LC-MS/MS for screening and confirmation has been increasing and this technique continues to be adapted by a rising number of labs, often replacing GC-MS assays. When any new confirmatory technique is implemented, debates arise regarding what constitutes a confirmation. Although it has been established that three ions are necessary for GC-MS SIM confirmation, criteria for an LC-MS/MS confirmation is still a highly debated topic. In this work, confirmation using two MRM transitions is compared and contrasted with confirmation using full scan linear ion trap MS/MS spectra.

Methods: Standards of various drug compounds were spiked into drug free urine at various concentrations and diluted 10x with mobile phase. Analysis was performed on an LC interfaced to a hybrid triple quadrupole/linear ion trap (LIT) mass spectrometer (Applied Biosystems 3200 QTRAP® System). All compounds were analyzed using positive mode electrospray ionization. For the MRM only method, two MRM transitions per analyte were monitored with the second transition functioning as a qualifier ion. The ratio of the peak areas of the target MRM to the qualifier MRM was calculated. For confirmation, it was required that the ratio be within +/- 20% of the standard. When full scan MS/MS spectra were used for confirmation, an MRM survey scan was used to detect the presence of an analyte. If an analyte was detected, the system automatically acquired a full scan MS/MS spectrum of the compound using Q3 operating in LIT mode. The resulting spectrum could be searched against a library for identification and confirmation. A purity match of 60% or higher was required for confirmation.

Preliminary data: Both MRM and LIT full scan MS/MS were sufficient for confirmation at mid-level concentrations. Differences were observed at the extremes – very low levels (10x below cutoff levels) or very high levels (>50x cutoff levels). Cutoff levels for most analytes were approximately 100 ng/mL with the exception of fentanyl, which was 1 ng/mL. Generally, LIT confirmation was more robust at very high levels. MRM confirmation worked better at very low levels as long as the qualifier ion had sufficient intensity. If the qualifier ion had low intensity, as was the case with certain amphetamines, LIT MS/MS yielded better confirmatory results.

Conclusion: Initial findings indicate that both the full scan method using a LIT and the MRM with two transitions method are robust and precise in performing confirmations. The ability to successfully confirm a drug identification depended on many factors, including qualifier ion intensity and analyte concentration.

Keywords: LC-MS/MS, drug screening, drug confirmation

P87. Determination of drugs in blood, performance monitoring over time by analysis of data from the QUARTZ proficiency testing scheme

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Introduction: The QUARTZ proficiency testing scheme is open to participants from any industry and is designed to promote quality and comparability in the measurement of a range of prescription and illicit drugs in human blood Samples of whole blood are spiked with one or more of an extensive list of drug and their metabolites, and distributed to participants for analysis. As with all proficiency testing schemes, an important aspect of their design is the monitoring and improvement of the quality of a laboratory's measurements over a period of time, which can assist in the evaluation of methods and instrumentation, educate laboratory staff and demonstrate the quality of results to third parties.

Methods: Using a range of statistical techniques the data produced by participants, over the past six years of QUARTZ, can be analysed. Various performance summary data is calculated to demonstrate where the overall performance of individual laboratories requires improvement, or where there are concerns regarding the measurement of a particular analyte. Individual laboratory performance, for a range of unidentified laboratories, will be analysed over time, using a range of statistical techniques.

Results: Over the period of six years up to 25 laboratories participated in 25 rounds of the QUARTZ proficiency testing scheme. The average percentage of satisfactory results as determined by z-scores was 81.19%, with a range of 43 to 100%, a well ordered group should have 90% percent satisfactory z-scores. Summary data shows a considerable range of performance for laboratories within the scheme. Analysis for a number of repeated common drugs demonstrated a consistent/improving performance for the participant group over the study period, whilst analytes where measurement was a concern could also be identified by the use of overall performance measures. Graphical monitoring of individual laboratory performance over time clearly indicates occasions when attention and remedial action are required.

Conclusions: The data produced by the participants from the QUARTZ proficiency testing scheme is a reasonably well ordered system, as can be seen by the overall percentage of satisfactory results. The group demonstrates consistent/improving performance when drugs are repeated within the scheme and summary data is a useful tool for identifying analytes or concentration levels of concern. Within the group however, summary data of results demonstrates that there is clearly a significant range of laboratory performance. Monitoring the performance of laboratories over time is a useful tool for identifying occasions when attention and remedial action are required.

Keywords: QUARTZ, drugs, analysis

P88. Determination of benzodiazepines in whole blood by ultra performance liquid chromatography tandem mass spectrometry

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Aims: An ultra performance liquid chromatography tandem mass spectrometry (UPLC-MSMS) method has been developed and validated for the determination of benzodiazepines marketed in Norway and used in impairment cases on forensic human whole blood samples.

Methods: The following compounds were included: alprazolam, clonazepam, diazepam, flunitrazepam, midazolam, nitrazepam, N-desmethyldiazepam (metabolite of diazepam), oxazepam and phenazepam. Aliquots of 500 µL whole blood were added 500 µL of borate buffer pH 11 and extracted by immobilized liquid-liquid extraction on ChemElut® columns (Varian) using 7.5 mL of tert-buthyl-methylether. Deuterated analogues were used as internal standards for all analytes, except for midazolam and phenazepam which had no commercially available deuterated analogues, and therefore used diazepam-d_5 and flunitrazepam-d_7 respectively. The analytes were separated using UPLC with a 2.1 x 100 mm BEH C_{18}-column, 1.7 µm particle size, and quantified by MS/MS using multiple reaction monitoring (MRM) in positive mode (Acquity UPLC-Premier XE MS/MS, Waters). Two transitions were used for the analytes and one transition for the deuterated internal standards. The run time of the method was 8 minutes including equilibration time.

Results: The concentration ranges for the benzodiazepines in the method span a broad range varying from the lowest concentration of 0.005 µM for flunitrazepam to the highest of 40 µM for oxazepam. The calibration curves of extracted whole blood standards were fitted by quadratic calibration curves with r^2 values ranging from 0.992 to 0.999. The relative standard deviations (RSD) of the between-day precision ranged between 4.3 and 13.7%. Recoveries of the analytes were from 82 to 99%. The LOQ were evaluated by extraction of blank whole blood containing various concentration levels of analytes (n=10). Blood from both living persons and autopsy cases were included. The LOQs for the analytes ranged from 0,004 to 0,160 µM. The robustness of the UPLC-MSMS method was found satisfactory using factorial design. Matrix effects were studied both by postcolumn infusion and postextraction addition and found to be between 82 and 120%. A comparison with other analytical LC-MS methods was performed during method validation by the analysis of approximately 100 authentic samples previously analyzed by the Division of Forensic Toxicology at the Norwegian Institute of Public Health. Good correlation was seen for all analytes.

Conclusion: This immobilized liquid-liquid extraction UPLC-MS/MS method has replaced a protein precipitation LC-MS-method, resulting in shorter runtime, better separation, cleaner extracts and improved specificity. In addition, the introduction of many deuterated internal standards makes the quantification more robust towards varying experimental conditions. The method has been used for routine forensic confirmation analysis for investigations of suspected impairment, primarily in DUI-cases, in approximately 1500 cases per February 2008.

Keywords: benzodiazepines, UPLC-MS/MS, whole blood

P89. Ethyl sulfate: previously thought to be totally stable against bacterial degradation – wrong!

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Objective: To test the bacterial degradation of ethyl sulphate, which is a marker for ethanol consumption complementary to "unstable" ethyl glucuronide.

Introduction: Ethyl sulphate (EtS) seems to have advantages over ethyl glucuronide (EtG) due to the reported unstability of EtG caused by bacterial urinary tract infection. Bacterial degradation has been reported in urine samples and in post-mortem samples for EtG but not for EtS. For testing the degradation, either samples can be stored under "degradation" conditions in tissues or body fluids spiked or contaminated with bacteria, or "bacterial degradation media" can be spiked with the analyte of interest: the latter one is a procedure to test biological elimination (or persistence) in environmental chemistry (using OECD tests). Bacterial glucuronidase activity can be tested using commercial test kits (with a glucuronidated substrate and detection of a coloured cleavage product). For sulfatase activity, no such test was available at the time of our investigations. We tested the stability of ethyl sulphate in a standardised Closed Bottle Test (OECD 301, low inoculum density), and in a standardised Oxitop-test (OECD 302, higher inoculum density).

Results: In the Closed Bottle Test ethyl sulphate was stable for 28 days, in the Oxitop test, ethyl sulphate was stable for 6 days, from day 6 to day 28, 84% of the added ethyl sulphate were degraded.

Conclusions: Depending on the type and concentration of bacteria, ethyl sulphate can be degraded by the incubation medium. This is of great importance for post-mortem toxicology, since the state of putrefaction is usually not defined according to the type or class and amount of bacteria present, but rather to visible signs of degradation of a corpse, besides colour, smell, post-mortem time until autopsy etc. For clinical urine samples, it should be considered, that bacterial infection can result in degradation of ethyl glucuronide, and under certain circumstances, the possibility of degradation of ethyl sulphate should be taken into consideration, too.

Preservatives, such as boric acid, sodium fluoride, chlorohexidine, sodium azide or thymol can be used for prevention of bacterial growth, and - although applied in rather high concentrations – still do not have to disturb analysis for EtG depending on the analytical system used. Examples for preservatives will be shown.

Keywords: ethyl sulphate, instability, bacteria

P90. Comparison of results randomly and routinely controlled Polish drivers

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Introduction: Epidemiological studies on drugs use among drivers have been started in Poland late summer of 2007. Randomly stopped drivers are subjected to oral fluid collection. The samples are next anonymously analysed in the laboratory. Results of first batch (more than 500 saliva samples) were compared with results obtained from blood analyses of routinely controlled as well as injured or killed in accidents drivers in the corresponding period. It should be noticed that in last both cases blood collection is undertaken by policemen or prosecutor on the basis of drugs abuse suspicion. That may cause high percentage of positive results in case of blood testing. The epidemiological research is conducted as a part of European project DRUID (Contract No TREN-05-FP6TR-S07.61320-548404-DRUID).

Methods: Blood samples taken from routinely controlled as well as injured or killed drivers were screened using ELISA method for six groups: opiates (morphine; cut-off [ng/mL] 20), cannabinoids (THCCOOH; 5), cocaine (benzoylecgonine, BZE; 50), amphetamines (amphetamine; 50), metamphetamines (MDMA; 50) and benzodiazepines (clonazepam; 10). Positive results of blood were confirmed using GC-MS, LC-MS or LC-MS/MS methods. Oral fluid samples were screened by the same chromatographic methods for the following drugs: morphine, 6-MAM, codeine, tramadol, methadone, amphetamine, metamphetamine, MDA, MDMA, MDEA, cocaine, BZE, alprazolam, diazepam, nordiazepam, flunitrazepam, clonazepam, lorazepam, oxazepam, carbamazepine, imipramine, zolpidem,

zopiclone, and THC. Samples of blood (0.2 mL) and oral fluid (0.5 mL) were subjected to LLE and SPE, respectively.The used methods had the following LODs and LOQs [ng/mL]: 1-5 and 1-25 for both specimens. Detailed validation parameters were published in TIAFT/ICADTS proceedings from Seattle.

Results: From routine control 36 out of 73 (49.3%) drivers were positive for one or several drugs. There were mainly THC (20.5%), amphetamine (16.4%), MDMA (6.8%), clonazepam (4.1%), and others, e.g. BZE, carbamazepine. Among injured or killed drivers 29 out of 114 (25.4%) were under the influence of THC (8.8%), amphetamine (6.1%), MDMA (1.8%), metaphetamine (0.9%), diazepam (2.6%), clonazepam (1.8%), lorazepam (0.9%), levomepromazine (0.9%), carbamazepine (0.9%), morphine (0.9%). In randomly stopped drivers only 8 out of 525 (1.52%) saliva samples were positive for THC (0.57%), amphetamine (0.38%), BZE (0.19%), tramadol (0.19%), oxazepam (0.19%).

Conclusion: At this stage of research only general conclusion could be draw. Policeman or prosecutor decision on blood collection from drivers taken on the basis of drugs abuse suspicion is the main reason of high percentage of positive results in case of blood testing. Pattern of abused drugs in all three groups seems to be similar, so the picture of randomly controlled drivers is right. The number of oral fluid samples that volume was lower than 0.3 mL was 38%. These samples were also analysed. Only 9 persons (1.7%) refused to take part in the research. The LODs (1-5 ng/mL) for benzodiazepines in the oral fluid is still insufficient.

Keywords: random control, saliva, drugs, drivers

P91. Alcohol in fatal traffic accidents in Finland

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Introduction: The Traffic Safety Investigations Teams in Finland were established in 1967 to produce traffic accident information, to evaluate risk factors and injury risks and make proposals for improvement. Those teams (20) cover the whole country and consist of 260 experts in different fields such as police, vehicle technology, road technology, medicine and psychology. The teams investigate all fatal traffic accidents and also a part of severe non fatal accidents. This function is statutory and independent. The Traffic Safety Investigation Teams work under official supervision of the Ministry of Traffic and Communication although they are part of the Traffic Safety Committee of Insurance companies.

Traffic flow surveys of National Public Health Institute, Helsinki University and Police have shown that every 600th (~0.15%) driver is a drunken driver (BAC more than 0.5‰) and 1 in 130 (~0.8%) of all tested drivers is in the limits 0<BAC<0.5‰. In the Finnish population of 5.2 million, about 25 000 drink drivers are caught every year by the police and about 1.7 million drivers are breath tested every year in police controls.

Almost 10 000 persons are injured annually on the Finnish roads. About 13% of those are casualties of drink driving accidents.

Methods: This survey is based on information from the years 1986-2006 released by road accident investigation teams, in cases where the blood alcohol content of a driver involved in an accident was at least 0.5‰.

Results: The road accident investigation teams have investigated annually about 270 accidents fatal to at least one motor vehicle driver or passenger. In 2005, 310 persons died and 224 were injured in those accidents. Every year, about 70 fatal traffic accidents have been caused by drink drivers. One fourth of all fatal traffic accident victims were killed in drink driving accidents. In fatal accidents, 4 in 5 drink drivers had a BAC level 1.2‰ or higher. In fatal motor vehicle accidents, the proportions of drink drivers were the highest in age groups 24 or under and from 25 to 44 years. In both age groups, the proportion of drink drivers was typically between 30 and 40 per cent. For sober drivers, the most common accident type was head-on collision. Contrastingly, drink drivers had a high proportion (65%) of run-off-road / single vehicle accidents.

Conclusions: Drink driving was often associated with speeding and misuse of safety belt (about 42% of drink drivers in fatal accidents). Drink drivers typically had several problems. Alcohol problems, recidivism of drink driving, previous accidents and other traffic offences were all common among drunken drivers. The proportions of drink drivers in fatal traffic accident cases have remained quite steady in the last twenty years.

Keywords: alcohol, fatal traffic accidents, traffic safety investigations teams

P92. THC and THCCOOH concentrations in blood, oral fluid and urine specimens taken from drivers and patients. Contribution to the interpretation of analytical results in forensic toxicology

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Introduction: Cannabis is still the most commonly used illicit drug in the world. In Poland among 243 drivers who caused road accident in 2001-2004, 26% were under the influence of THC alone and 16% of THC and amphetamines. The measured concentrations [ng/mL] of THC and THCCOOH in blood samples taken from those drivers ranged from 1 to 20 and 8-168, respectively. This study was aimed at determining disposition of THC and/or THCCOOH in different biological fluids.

Methods: Three sources of materials were analysed. (1) Blood and saliva samples taken simultaneously from hospitalised drivers (n=12) at admission (0 h) to hospital and after 24 h of hospitalisation, and time of the last administration of cannabis was not known. (2) Blood, saliva and urine samples collected at three sampling points (0 h and 24 h of hospitalisation, and 12 h after last smoking) from patients (n=9). (3) Blood (117) and urine (n=51) samples taken from drivers involved in road incidents (routine control or accident). In this group the blood samples from 5 drivers were collected every hour for three hours. Concentrations of THC and THCCOOH in biological fluids were determined by GC-MS-NCI method after LLE or SPE. The SPE method was used after enzymatic hydrolysis for urine samples. Both methods had the following validation parameters [ng/mL]: LOD – 0.25, LOQ – 0.5, LOL – from LOQ up to 20 for oral fluid and blood samples and to 100 for urine samples for both analytes.

Results: In all analysed groups the concentrations (expressed as mean±SD, range and ng/mL) of THC and THCCOOH varied a lot. In the first group at admission to hospital THC blood concentration was 8.5±7.1 (range: 0.5-26.4), and after 24 h these values were 2.7±3.0 (0.5-15.3). The THC oral fluid concentrations were 7.0±8.9 (0.5-32.5) and 1.6±2.4 (0.5-10.4) at the respective sampling points. The saliva/blood THC concentration ratio was 0.82 at admission and 0.60 after 24 h of patient hospitalisation. The concentrations of THCCOOH in blood at 0 h and 24 h sampling points were 49.2±74.4 (0.5-421) and 38.1±56.2 (0.5-291). In the second group of patients concentrations of THC and THCCOOH were the highest at admission and ranged for THC 0.8-3.5 in blood, 0.5-10.6 in oral fluid and for THCCOOH 4.8-15.9 in blood and 0.3-608 in urine. After 12 h since last smoking THC concentrations in blood and oral fluid did not exceed 2.0 and 5.6, respectively. Among drivers involved in road incidents the concentration of THC was 5.0 (0.5-17.3) and THCCOOH 42.0 (3.2-286) in blood, and 137.8 (3.5-894) in urine. 69% of the measured concentrations were below the legal analytical limit of 2 ng/mL. In the blood samples collected every hour for three hours the concentrations of THC fell to between 50% of initial values and THCCOOH increased to 300%.

Conclusions: THC can be detected both in oral fluid and blood up to 24 h following last smoking. Within 12 h blood THC concentrations declined below 1 ng/mL. Simultaneous determination of THC and THCCOOH in

blood samples collected at several 1h intervals can be stronger evidence of last cannabis use even at low measured THC concentrations in blood.
Keywords: THC concentrations, biological fluids, determination

P93. False positive ethyl glucuronide resulting from incidental alcohol exposure

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Introduction: Ethyl Glucuronide (EtG) is a novel biomarker of alcohol consumption. It is a water soluble, stable, non-volatile, direct metabolite of ethanol accounting for between 0.5 and 1.5% of the total ethanol elimination. Whilst the detection period for alcohol is relatively short, EtG can be detected for up to 80hrs and peaks at approximately 4hrs. It is a direct, specific and sensitive marker of acute alcohol consumption. It is not influenced by age, gender, medication or non-alcohol related disease and is not dependant on chronic alcohol abuse, unlike other conventional biomarkers. For these reasons it is proving a promising biomarker for monitoring alcohol abstinence. Such examples include liver transplant recipients, recovering alcoholics in withdrawal treatment programmes and some fields of employment, such as in aviation. However, ethanol is not only contained in alcoholic beverages. It can be found in a wide range of every day products, including; foods, medicines, mouthwashes, perfumes, disinfectants, hand sanitisers and automotive fuel. The alcohol in these products may enter the body via oral consumption, absorption or inhalation. We investigated the effect of incidental alcohol consumption, resulting from oral hygiene products and cough syrup, on urinary EtG concentrations.
Methods: A total of 7 alcohol containing oral hygiene products (6 mouth washes and 1 breath freshener spray) and two cough syrups were purchased and used as directed on successive days. The dose ranged from 10-20mL, duration of use from 30 to 60sec and alcohol content from 2.3-23% v/v. Urine samples were collected from a 28 year old female volunteer before use (blank) and 4hrs (±15min) post dose. Subsequently, additional samples (3+1) were obtained under identical conditions from a further two female volunteers of similar age. EtG was measured using the Microgenics DRI® EtG Enzyme Immunoassay on the Olympus AU400 platform. Assays were semi-quantitative (0, 100, 500, 1000, 2000ng/mL) with 4 QC levels employed (375, 625, 750, 1250ng/mL). The sensitivity of the assay was quoted as being 15.3ng/mL, but cut offs of 500ng/mL or 1000ng/mL are typically recommended. Where possible EtG results were corrected for the effect of internal dilution using creatinine, measured using the Jaffe reaction on the Siemens Advia 2400 and were compared to ethanol concentrations measured by head Space GC-FID on the Shimadzu GC 2014.
Results: All initial test and control samples were negative for both ethanol and EtG (<100ng/mL). From the subsequent tests, one volunteer yielded results similar to the initial test subject the other produced EtG concentrations of 370, 226 and 174ng/mL or 64, 40 and 28ng/mmol creatinine respectively for 3 different alcohol containing mouth washes.
Conclusion: Further work is required to establish the extent of false positives resulting from incidental alcohol exposure, such as from foods, medicines, hygiene products and disinfectants. This is of particular importance when alcohol abstinence is required for drivers to regain their licences following a charge of driving impairment, where liver transplantation is conditional on abstinence and where the loss of employment, child custody or other privileges may result.
Keywords: ethyl glucuronide, false positive, mouth wash

P94. Calculation of carbohydrate deficient transferrin by using different methods (%CDT vs Tf index): critical considerations

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Introduction: Carbohydrate Deficient Transferrin (CDT) is a worldwide recognized biochemical marker of chronic alcohol abuse and for this reason is widely used in different clinical and forensic contexts. The determination of CDT is currently based on immunoassays, chromatography and capillary electrophoresis, each method having specific advantages and disadvantages. A general consensus states that immunoassays are suitable for screening purposes whereas chromatography/electrophoresis are most suited for confirmation. Lacking an international standard of CDT, the results are currently expressed as percent ratio of CDT isoforms/total transferrin (Tf) (% CDT). However, several authors still express CDT concentrations as the percent ratio of disialo-Tf/tetrasialo-Tf (Tf index) [Tagliaro F. *et al*, Forensic Sci Review 2000;12:133-149]. In view of a standardization of CDT determination, the two calculation methods have been applied in either HPLC and capillary electrophoresis (CE) experiments and compared in order to identify their advantages and/or disadvantages.
Methods: serum samples from 50 subjects including both alcohol abusers and normal subjects have been analysed with validated HPLC and CE methods [Bortolotti F. *et al*, Clin Chem. 2005;51:2368-2371]. The chromatograms/electropherograms have been analysed using the valley-to-valley integration mode. Peak areas have been used for calculation of CDT concentration using the following equations: i.) % CDT=(asialo-Tf +disialo-Tf)/ (asialo-Tf +disialo-Tf+trisialo-Tf+tetrasialo-Tf+pentasialo-Tf); ii.) Tf index=disialo-Tf/tetrasialo-Tf.
Results: the CDT values calculated with the different methods have been compared by using the least square regression method with the following results.
HPLC: Tf index (x) *vs* %CDT (y): y=0.8106x+0.0463 (R^2=0.9973);
CE: CDT index (x) *vs* %CDT (y): y=0.8119x+0.0595 (R^2=0.9978).
The CDT results calculated either as %CDT or as Tf index from HPLC and CE have also been compared with the least square regression method with the following results.
% CDT: CE (x) vs HPLC (y): y=0.8567x+0.4291 (R2=0.8693);
Tf index: CE (x) vs HPLC (y): y=0.8583x+0.5419 (R2=0.8768).
Conclusion: Notwithstanding an obvious difference between %CDT and CDT index related to the higher denominator in the calculation of %CDT, an excellent correlation between the two expression modes has been found. This can be explained by a negligible contribution of trisialo- and pentasialo-Tf peaks on the overall transferrin area calculation. Consequently the correlation between CDT results from CE and HPLC methods is not affected by the difference in the CDT expression mode.
Keywords: carbohydrate deficient transferrin, Tf index, % CDT

P95. A simplified method for the analysis of ethyl glucuronide (EtG) in whole blood

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We present a validated, simplified method for measuring EtG in post mortem blood.
Method: 1.2mL of methanol was added to 0.6 mL of whole blood, vortexed for 30 seconds and frozen for 20 minutes at –20°C. The samples were centrifuged at 1159G for 5 minutes and the supernatant dried down at 60°C. The residue was re-suspended in 1 mL of 1% formic acid in H_2O and vortexed for 15 seconds. The samples underwent an SPE clean up using 200mg Clean Screen extraction columns. The samples were run on the 3200 QTRAP LC/

MS/MS using a Phenomenex Gemini 105mm x 2mm x 5µm C18 column with mobile phase 95:5. 0.1% formic acid in water: 0.1% formic acid in acetonitrile. The EtG parent ion (221.2) with 2 qualifier ions (85 and 74.9) were used for quantification. Pentadeuterated-EtG (parent 226.2 and qualifier 85.1) was used as an internal standard. Retention time for both EtG and pentadeuterated-EtG was 1.99 minutes. The method was validated in the range 0.05mg/L to 10mg/L. Linearity was r=1.0000. The LOD was determined as 0.029mg/L (signal: noise ratio >3:1) and LOQ as 0.1mg/L, (signal: noise ratio >10:1). EtG recovery was 110% of pentadeuterated at 5mg/L. Intra-day accuracy at 1mg/L was 95.5-104.4% and 5mg/L 97.2-102.2%. Inter-day accuracy at 1mg/L was 89.6 – 108.9% and 5mg/L 85.8-107.5%. Intra-day precisions at 5mg/L and 1mg/L (n=9) were 1.55% and 3.15% respectively. Inter-day precision, were 6.83% and 8.39% respectively. Stability studies over 28 days showed average decreases of 3.5% (-20ºC), 4.2% (4ºC) and 7.2% (23ºC). Clinical validation was performed on post mortem blood samples. Analysis was either at request of, or following permission for sample use by Her Majesty's Coroner.

Clinical sample groups: *High alcohol consumption:* 23 subjects with post mortem blood alcohol >200mg/100mL; with equal or higher urine alcohol concentrations. There were no reports of putrefaction or visceral damage, and no products of putrefaction (phenethylamines) detected. *Low level alcohol consumption:* there was history fulfilling the criteria of the high alcohol consumption group, but with a blood alcohol concentration of <100mg/100mL, and vitreous or urine alcohol of less than 100mg/100mL. *Low blood alcohol levels and products of putrefaction*: 10 cases in which there was no history of alcohol consumption, a blood alcohol of 100mg/100mL, no alcohol detected in either urine or vitreous and evidence of phenethylamine (but no amphetamines). *No alcohol consumption suspected*: 11 aged children aged <10 with no suspicion of alcohol consumption.

Results: The high alcohol consumption group had a mean blood alcohol concentration of 284mg/100mL. The EtG concentration ranged from 1.3 to 21mg/L (mean 4.8mg/L). The "low level alcohol consumption" group mean blood alcohol concentration was 32mg/100ml.The EtG concentration ranged from 0 to 1.6mg/L (mean 0.37mg/L). The "low blood level alcohol + evidence of putrefaction" group had a mean blood alcohol of 26mg/100mL. EtG was not present in any of these cases. There was no statistical difference in alcohol levels between the 2 low alcohol groups with blood alcohol less than 100mg/100mL blood, but a statistical difference ($p<0.01$) in the EtG concentrations.

In the children, where alcohol was not detected in any case, there was no EtG detected.

Conclusion: This simple method allows appropriate detection of EtG, with good distinction in the problematic area for post mortem interpretation of blood alcohols less than 100mg/100mL.

Keywords: EtG, alcohol, LC-MS/MS

P96. Driving under the effects of drugs of abuse based on studies of oral fluid

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Introduction: Oral fluid as sample to determine the use of drugs of abuse is being used and took into account by different governments in order to improve traffic security. Thus, the objectives of this study include the evaluation of the use of drugs of abuse ratio in leisure environments, in order to determine which drugs are the most widely used, which are normally used in poly-consumption and which are usually mixed with alcohol.

Methods: Total sample rise to 216 cases which were collected on the road by traffic police and analyzed by an on-the-road driving test of possible consumption. Positive samples has been duplicated in a tube of assay, stored at 4ºC and later analyzed by GC-MS/MS. The identified analytes by the immunoassay test have been Δ^9-THC, 6-monoacetylmorphine, cocaine, amphetamine and methamphetamine. Cut-off values are 31 ng/mL for Δ^9-THC, 100 ng/mL for cocaine and 50 ng/mL for 6-acetylmorphine, methamphetamine and amphetamine.

The confirmation method has been GC/MS-MS. Sample preparation consists in the addition of 1mL of oral fluid and 1mL of phosphate buffer at pH 6 with 20 µL of cocaine-d3, 6-MAM-d3, amphetamine-d5, methamphetamine-d9 and THC-d9 for a final concentration 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 since dryness and derivatized with 40 µL of BSFTA-TMCS at 80ºC for 20 min. or PFPA for amphetamines and methamphetamines at 50ºC for 40 min. 2 µL of the sample are injected in a split-less GC/MS/MS. A temperature program is carried out starting at 90ºC for 1 min followed by a ramp of 20ºC/min since 240ºC and a second ramp of 5ºC/min since 300ºC by using a column VF-5ms 30mx0.25mm. Detector used has been a Varian ion trap with electron impact ionization considering the MS-MS alternative with the father ions, 386 for Δ^9-THC-TMS, 182 for cocaine, 399 for 6-MAM-TMS, 204 for methamphetamine-PFP and 190 for amphetamine-PFP The limits of detection (LOD in ng/mL) and quantification (LOQ in ng/mL) respectively for the confirmatory analysis are 2.5 and 10 for Δ^9-THC and cocaine; 5 and 20 for 6-acetylmorphine, methamphetamine and amphetamine. Precision and accuracy values have not been measured in the first phase of the study. The confirmed analytes in order to consider the test as a valid one are Δ^9-THC, cocaine, 6-acetylmorphine, amphetamine and methamphetamine (MDMA and MDEA with a cut-off value of 50 ng/mL for both analytes).

Results: It was detected positive samples for the studied drugs (cocaine, opioids derived from heroin (6-MAM), amphetamines/methamphetamines and Δ^9-THC) in a 73.1% of the total sample (216). Sample shows a high degree of poly-consumption of different drugs differently distributed depending on one single drug detected, 64.5%, two drugs, 30.4%, or three or more drugs, 5.1%. The most widely detected drug of abuse as a single consumption has been THC (cannabis) with a 51% while cocaine has a 48%. The rest of the drugs are always mixed. The grater combination in poly-consumption is THC-Cocaine reaching an 89.5% of these cases.

Conclusions: It is important to notice the high ratio of positive results in drivers, although it has to be taken into account that it is a slanted sample in reference with collection time (at night/early morning), with the kind of vehicle and driver (car-apparent age 20-40 years) and with the area (leisure zones). However, the tendency to poly-consumption of drugs of abuse mixed with alcohol is confirmed in leisure zones and the later use of vehicles under drug effects. Thus, it is necessary to consider the mixed/alternated use of ethilometers and on-the road detection kits of drugs of abuse in oral fluid to support the traffic security.

Keywords: drugs of abuse, saliva, legal medicine

P97. Oral fluid test validation: Results confirmation by GC-MS/MS analysis

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Introduction: The rising number of traffic deaths related to the use of drugs of abuse has made indispensable the use of simple on-the road tests in an easy and fast way. The used test in Spain is immunoassay-based oral fluid test. Due to the great number of analysis, it has become essential the validation of this test by using more sensible methods which allow to determine not only the positives and negatives ratios but also the ratio of false positives and negatives showed by this test. Accordingly to this, it has been decided a test validation by analysis by gas chromatography and detection by mass spectrometry (GC-MS/MS).

Methods: Sampling was carried out in traffic control done by the police collecting duplicated samples in all of those cases in which the driver seemed to be under the effects of drugs. On one side it was *in situ* analyzed by the

oral fluid test, and, on the other side, a second sample was collected in a sample collector, stored at 4ºC and lately analyzed by GC-MS/MS.
The identified analytes by the immunoassay test have been Δ^9-THC, 6-monoacetylmorphine, cocaine, amphetamine and methamphetamine. Cut-off values are 31 ng/mL for Δ^9-THC, 100 ng/mL for cocaine and 50 ng/mL for 6-acetylmorphine, methamphetamine and amphetamine. The confirmation method used has been GC-MS/MS which has a limit of detection established in the same cut-off of the immunoassay test according with the objective of a test validation with a known cut-off. The confirmed analytes in order to consider the test as a valid one were Δ^9-THC, cocaine, 6-monoacetylmorphine, amphetamine and methamphetamine (MDMA and MDEA with a cut-off value of 50 ng/mL for both analytes).
The reason of using a MS-MS method is to determine the possible cross-reactions and to identify which analyte reacts in the immunoassay test. For GC-MS/MS analysis, sample preparation consists in the addition of 1ml of oral fluid and 1ml of phosphate buffer at pH 6 with 20 µL of cocaine-d3, 6-MAM-d3, amphetamine-d5, methamphetamine-d9 and THC-d9 for a final concentration 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 since dryness and derivatized with 40 µL of BSFTA-TMCS at 80ºC for 20 min or PFPA for amphetamines and methamphetamines at 50ºC for 40 min. 2 µL of the sample are injected in a split-less GC-MS/MS. A temperature program is carried out starting at 90ºC for 1 min followed by a ramp of 20ºC/min since 240ºC and a second ramp of 5ºC/min since 300ºC by using a column VF-5MS 30m x 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 Δ^9-THC-TMS, 182 for cocaine, 399 for 6-MAM-TMS, 204 for methamphetamine-PFP and 190 for amphetamine-PFP. Total size of the sample has been 216 cases. No studies of limits of quantification or linearity have been done; owing the first step of the study was qualitative.
Results: From the 216 cases analyzed by using the oral fluid test, it has been obtained 155 positive and 61 negative results. In the analysis by GC-MS/MS 158 positive and 58 negative results were obtained. From the 61 negative results, 19 were confirmed, showing 6 false negatives, and 3 false positives from 155 positives confirmed. Both methods show a concordance of the 98.1% of the positive cases and 68.4% of the negatives cases.
Conclusions: Results allow to consider the oral fluid test as validated owing that it shows a concordance ratio with GC-MS/MS higher than a 95%. This test could be rejected for Δ^9-THC (30% error in cannabis negative samples). Results let consider oral fluid test as a good enough method for the detection of narcotic substances *in situ*, though later confirmation is highly suggested by using a more sensible method.
Keywords: oral fluid test, saliva, legal medicine

P98. Development of a fast one-step on-site oral fluid drug screen

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Introduction: Oral fluid drug screen has been gaining acceptance due to its ease of obtaining specimen and the minimization of adulteration possibility. An on-site oral fluid test device, Oratect®III, was introduced to use a one-step collection and testing procedure to provide results for 7 drugs within 7 minutes utilizing 0.5 mL. of oral fluids. Feedbacks from roadside drug testing programs have shown high interest in a faster oral fluid drug screen. Here, we report the development of such a device – OratectXP™1.
Methods: The Oratect®III device housing was first redesigned to accommodate the testing of 5 drugs (Opiates, Methamphetamine, MDMA, THC and Cocaine) in a single test strip using 0.25 mL of oral fluid. Changes were then made in some of the raw materials for the test strip to enhance liquid flow. Each drug test was then reformulated to produce lower cut-off concentration. Studies were then undertaken to investigate the oral fluid collection time required to initiate the test, the time needed to obtain a stable result after the test had been initiated and the correlation of the test results with LC/MS results.
Results: Reproducible cut-off concentrations were obtained for both opiates and cocaine at 10 ng/mL. Methamphetamine and MDMA cut-offs were at 12.5 ng/mL. and Δ9-THC at 10 ng/mL. Collection time study on 200 donors showed a medium time of 60 sec. (range: 19 sec to 120 sec). This compares to the medium collection time of 150 sec. for Oratect®III. Test line intensities reached optimal levels by 3 min. for all tests in this new device as compared to 5 min with Oratect®III. Initial study of limited number of subjects has shown the confirmation of OratectXP™1 results by LC/MS ranged from 85 to 100% for the 5 drugs.
Conclusion: OratectXP™1 is demonstrated to be a fast oral fluid drug screen. A total time for both collection and testing is 5 min as compared to 7.5 min. for Oratect®III.
Keywords: oral fluid, drug screen, Oratect

P99. Verification of benzoylecgonine in extracted oral fluid samples with immunoassay

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Introduction: Benzoylecgonine, the main metabolite of cocaine, is known to extract poorly using most methods of liquid-liquid extraction. For analysis of drugs of abuse in oral fluid when liquid-liquid extraction is preferred, this important metabolite can be determined from the extract. Poor yields indicate however that most of the substance, if present, will remain in the aqueous phase. For screening of oral fluid samples where a low occurrence of positive cocaine cases are expected, e.g. prevalence and epidemiological studies and workplace testing in a general population, we present a screening for benzoylecgonine in oral fluid after liquid-liquid extraction using immunoassay. Two different techniques were optimised and tested for this purpose. The samples tested were oral fluid samples collected with the Statsure Saliva Sampler™. The target cut-off for the analysis was 10 ng/mL in neat oral fluid.
Methods: Two commercial reagent kits for detecting benzoylecgonine in urine were tested, EMIT® II Cocaine Metabolite Assay (Dade Behring) and CEDIA® Cocaine Assay (Microgenics). The instrument used was a Vitalab Viva EMIT® device (Dade Behring). The parameters were optimised separately for both kits: volume of antibody 110 µL, volume of reagent 180 µL and measurement at wavelength 340 nm for EMIT® and volume of antibody 110 µL, volume of reagent 181 µL and measurement at wavelength 578 nm for CEDIA®. The sample volume used for measurement was 30 µL.
The samples tested and calibration samples were prepared in spiked oral fluid. A blank sample, low positive control (4.54 ng/mL) and a mid concentration sample (9.1 ng/mL) were measured in each run. The spiked samples were prepared by adding a solution of benzoylecgonine (in MeOH) in appropriate volumes to oral fluid. The aqueous phase of the samples after liquid-liquid extraction was mimicked by an aliquot of 0.5 mL of the spiked sample, 0.5 mL of the buffer from the Statsure Saliva Sampler™ and 0.1 mL of borate buffer. All samples tested were prepared in this dilution.
Results: The lowest concentration at which the difference between the absorbance was sufficient for qualitative determination of benzoylecgonine was found to be 2.72 ng/mL. This is below the target of 4.55 ng/mL that the dilution of an oral fluid sample of 10 ng/mL gives. The reproducibility of the calibration was tested by repeating measurements of blank, low positive level samples, and samples of 9.1 ng/mL. The variation of differences of the absorbances between the levels was below 30.5% (RSD%) for all levels and both reagents. Cross reactivity was tested with 130 substances at various levels. Only cocaine and ecgonine methyl ester gave positive results, showing

a good specificity. Samples at the low positive level and blanks in 10 replicates were tested on three different days and the variations in absorbance within and between the days were low. All blank samples were negative at all times and all low positive level samples were positive, indicating excellent sensitivity and accuracy. The stability was tested by measuring the same samples left on the auto sampler tray at room temperature and no decrease in performance was observed.

Conclusions: This presentation shows that screening for cocaine in the aqueous phase of an oral fluid sample after liquid-liquid extraction is possible with immunoassay and modified conditions of commercially available reagent kits for urine. The benefits of this procedure for screening for the presence of the cocaine metabolite benzoylecgonine are the possibility to use the waste fraction of samples extracted for other analyses, a labour efficient method and possibilities of automation of large sample quantities.

Keywords: oral fluid, benzoylecgonine, immunoassay

P100. Simultaneous determination of amphetamine-type stimulants and cannabinoids in fingernails by gas chromatography/mass spectrometry

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Aims: A gas chromatography/mass spectrometric (GC-MS) method was developed and validated for the simultaneous detection and quantification of four amphetamine-type stimulants (amphetamine (AP), methamphamine (MA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA)) and two cannabinoids (Δ^9-tetrahydrocannabinol (Δ^9-THC) and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH)) in fingernails.

Methods: Fingernail clippings (30 mg) were washed with distilled water and methanol, then incubated in 1.0 M sodium hydroxide at 95°C for 30 min. The compounds of interest were isolated by liquid-liquid extraction followed by derivatization with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) at 70°C for 15 min. The derivatized compounds were analyzed by GC/MS in the selective ion monitoring (SIM) mode.

Results: The linear ranges were 0.1-15.0 ng/mg for AP, 0.2-15.0 ng/mg for MDA, Δ9-THC and THCCOOH, and 0.2-30.0 ng/mg for MA and MDMA, with good correlation coefficients (r^2> 0.9991). The intra-day, inter-day, and inter-person precisions were within 10.6%, 6.3% and 5.3%, respectively. The intra-day, inter-day and inter-person accuracies were between -6.1 and 5.0%, -6.2 and 5.7%, and -6.4 and 5.6%, respectively. The limits of detection (LODs) and quantification (LOQs) for each compound were lower than 0.056 and 0.19 ng/mg, respectively. The recoveries were in the range of 74.0-94.8%. Positive GC/MS results were obtained from specimens of nine suspected MA or cannabis abusers. The concentration ranges of MA, AP, and THCCOOH were 0.92-1.41, 0.08-2.64 and 0.15 ng/mg, respectively.

Conclusion: Based on these results, the method proved to be effective for the simultaneous qualification and quantification of amphetamine-type stimulants and cannabinoids in fingernails.

Keywords: amphetamine-type stimulants; cannabinoids; GC-MS; fingernails

P101. Preanalytical stability of benzodiazepines on an oral fluid sampling device

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Introduction: In the last years, great efforts have been made in the development of new oral fluid testing systems. For some questions, oral fluid is considered to be a smart alternative to the common blood or urine testing, especially in roadside- or workplace drug testing. As most of the oral fluid sampling will take place in the field, the oral fluid collecting device and the testing system has to fulfill requirements such as robustness and easy handling. Besides, a valid sample analysis, with respect to analytical and forensic standards, such as the possibility of an independent confirmatory analysis of a test sample, has to be guaranteed. The presented study is dealing with the question of pre-analytical stability of eleven frequently abused benzodiazepines (bromazepam, chlordiazepoxide, clonazepam, diazepam, flurazepam, flunitrazepam, lorazepam, midazolam, nordazepam, oxazepam and temazepam) on such an oral fluid collecting device (Dräger DCD 5000). The collection device was designed to fit a special mobile testing system but it can also be sent to a laboratory for further confirmatory analysis.

Methods: Oral fluid from five healthy volunteers was pooled and spiked with 15 ng/mL of each analyte (except diazepam with a concentration of 30 ng/mL). To simulate possible sample shipping, 450 µL spiked oral fluid was given on a collection device and stored in the dark from 5 minutes up to 14 days at ambient temperature in a plastic tube. The collection device was simultaneously stored without further treatment after oral fluid collection ('native') and with addition of 950 µL of methanol, respectively. Additionally, a methanolic solution of benzodiazepines and spiked oral fluid with the same concentrations were stored. Repeat determination was carried out for every sample. After the storage time, 950 µL of methanol were added to 'native' stored collection devices. The oral fluid was removed from the collection device by centrifugation. Afterwards, the oral fluid/methanol mixture was concentrated under a gentle stream of nitrogen and the benzodiazepines were extracted using an alkaline liquid extraction. For quantitative analysis we used a modified version of our validated standard LC-MS/MS method for the detection of benzodiazepines in serum.

Results: We found different levels of loss of analytes due to degradation and/or adsorption to the collection device during the 14 days of 'native' storage. Nordazepam, temazepam, oxazepam, lorazepam, flurazepam and bromazepam showed recoveries better than 80% after 7 days of storage, but we also found massive loss of analyte, especially for benzodiazepines containing a nitro-group such as flunitrazepam and clonazepam. Loss of analytes could be prevented almost completely by methanolic storage of the collection device after sampling: After 14 days of methanolic storage, also clonazepam and flunitrazepam (nitro-group benzodiazepines) could be found at the same levels as on day one.

Conclusions: For confirmatory analysis of an oral fluid sample, we recommend the centrifugation of the collection device prior to sample shipping. If this should not be possible, addition of methanol immediately after sample collection is necessary to avoid degradation of some benzodiazepines (clonazepam and flunitrazepam) during shipment.

Keywords: oral fluid, pre-analytical stability, benzodiazepines

P102. Automated immunoassay for the detection of PCP in oral fluid on Roche instrument platforms

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Introduction: A homogeneous immunoassay is in development for the semi-quantitative and qualitative determination of PCP 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 other bodily fluids, and adulteration is more difficult.

Methods: The assay utilizes KIMS technology (Kinetic Interaction of microparticles in Solution) with liquid reagents that do not require reconstitution. The two-reagent system consists of a reagent containing a PCP monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as

the second reagent. The PCP oral fluid assay utilizes a cutoff of 1 ng/mL when using the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The test range of the assay is 0-8 ng/mL.

Results: When run in a semi-quantitative mode with a 6-point calibration on a Roche/Hitachi 917 analyzer, control samples at concentrations of 0.75 and 1.25 ng/mL show mean recoveries of 0.75 ng/mL (100%) and 1.26 ng/mL (101%), respectively. Within run precision studies (n=21) show %CV values of 6.9% and 5.9%, respectively, for these control levels. The measured lower detection limit (LDL) of the assay is 0.2 ng/mL (mean+2 standard deviations). The assay is specific to PCP with cross reactivity of less than 0.1% to dextromethorphan.

Conclusion: In summary, the assay produces accurate and reliable results and is well suited for routine screening of PCP in oral fluids.

*These assays are currently in development and have not been approved for use in the US by the FDA. Intercept is a trademark of Orasure Technologies, Inc.

Keywords: oral fluids, PCP, immunoassay

P103. Clinical evaluation of a new method for the detection of benzodiazepines in oral fluid

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Objective: The Dräger DrugTest 5000™ has recently emerged as a new diagnostic tool for rapid detection of drugs of abuse from oral fluid. The system comprises a disposable test-kit incorporating sampling unit and multiple immunochemical sensors as well as an optical analyzer for processing and interpreting sensor signals in less than ten minutes analysis time.

This study presents the evaluation of the Dräger DrugTest 5000 in terms of sensitivity, specificity, and accuracy of detection Benzodiazepines in oral fluid samples.

Method: Benzodiazepines were monitored by the Dräger DrugTest 5000 in a mixed population of workplace volunteers and volunteers being involved in a methadone substitution program (n=109). 10 frequently abused benzodiazepines (nordazepam, diazepam, oxazepam, temazepam, lorazepam, flunitrazepam, clonazepam, bromazepam, midazolam, and flurazepam) were subsequently determined during laboratory confirmatory analysis. After execution a direct indicating Dräger DrugTest 5000, each volunteer additionally sampled a Dräger DCD 5000™ oral fluid collection device. This oral fluid sample collector is shown to be suitable and analytically reliable as collection device for the laboratory confirmation of benzodiazepines; see reference. Collection devices were stored in 950 µL of methanol immediately after sampling und sent to a laboratory for confirmatory analysis. After ultrasonic treatment, the oral fluid/methanol mixture was removed from the collection device by centrifugation. Afterwards, the fluid/methanol mixture was concentrated under a gentle stream of nitrogen and the benzodiazepines were extracted using an alkaline liquid extraction. Quantitative analysis was performed using liquid chromatography tandem mass spectrometry. The limits of detection (LOD) for all benzodiazepines ranged from 3 ng/mL to 5 ng/mL (S/N≥4).

Results: Benzodiazepines were exclusively prevalent (35%) in the methadone substitution population (n=58). In the workplace population, all samples were detected negative for Benzodiazepines. With respect to the confirmational cutoff (7.5 ng/mL) clinical sensitivity of Dräger DrugTest 5000 for Benzodiazepine was 90% associated with two false negative samples. Moreover clinical specifity achieved 99%, obtained from one false positive sample, only. The accuracy of the Dräger DrugTest 5000 for benzodiazepines gave 97%.

Conclusions: Good correlations between the readings of the Dräger DrugTest 5000 for Benzodiazepines in oral fluid and the results of confirmatory analysis using LC-MSMS can be concluded. Due to the wide diversity of benzodiazepines the false positive sample might be explained by indetermined benzodiazepines. Thus, the clinical performance of the device proved to be competent and therefore suitable for use in diagnostic application for benzodiazepines.

P104. Determination of new generation antidepressants in human post-mortem blood, brain tissue and hair using a gas chromatographic-mass spectrometric method in positive chemical ionization mode

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Introduction: Although the new generation antidepressants (ADs) have a low toxicity profile, screening of blood and tissue samples in forensic cases is of interest as ADs are often involved in intoxications. ADs are frequently used together with other legal or illegal drugs and this can result in synergy of symptoms, a changed drug concentration due to inhibition of cytochrome P450 isoenzymes, or in a severe interaction such as the serotonin syndrome.

Objective: To develop and validate a solid phase extraction and a GC-MS method in PICI mode for simultaneous determination of ADs and their active metabolites in post-mortem human blood, brain tissue and hair.

Methods: A HP 6890 GC-5973 MSD was used in SIM for the quantification of mirtazapine, viloxazine, venlafaxine, citalopram, mianserin, reboxetine, fluoxetine, fluvoxamine, sertraline, maprotiline, melitracen, paroxetine, norfluoxetine, m-cpp, desmethylmianserin, desmethylmirtazapine, desmethylsertraline, desmethylcitalopram, and didesmethyl-citalopram. Fluoxetine d_6, mianserin d_3 and paroxetine d_6 were used as I.S.'s. The GC was equipped with a splitless auto-injector at 300°C and a 30m x 0.25mm i.d., 0.25-µm J&W-5ms column. The initial temperature was set at 90°C for 1 min, ramped at 50°C/min to 180°C for 10 min, and ramped again at 10°C/min to 300°C (5 min), with a constant helium flow of 1.3 mL/min. The MSD temperatures were 300°C for the transferline, 150°C for the quadrupole and 250°C for the CI-source. Methane was used as reagent gas at a flow of 1 mL/min.

Results: Sample preparation consisted of a strong cation exchange mechanism and derivatisation with heptafluorobutyrylimidazole. The method was previously described in plasma [Wille SMR et al. J Chromatogr A. 2005; 1098: 19-29] and was successfully adapted for blood, brain tissue and hair samples. Most ADs, as well as their heptafluorobutyryl derivatives were stable under different storage conditions. Matrix matched calibrators (n=6) ranged from subtherapeutic till high therapeutic concentration. Calibration curves were fit to a quadratic curve for PICI, with a weighting factor of $1/x^2$. A strong cation exchanger resulted in reproducible recoveries (73-106% for blood, 51-99% for brain) at three different concentration ranges (n=6). Intra- and inter batch precision at LOQ (5-25 ng/mL blood; 25-62.5 ng/g brain), low, medium and high concentrations fulfilled the criterion of a relative standard deviation below 20% at LOQ and below 15% at the other concentrations for most compounds. Accuracy ranged from 71-117%. Sertraline, fluoxetine, and citalopram and their metabolites were quantified in 4 post-mortem cases. Blood concentrations ranged from subtherapeutic till toxic concentrations, while brain/blood ratio's ranged from 0.8 till 17. Hair concentrations ranged from 0.4 till 2.5 ng/mg dependent on the compound and hair segment.

Conclusion: The developed GC-MS method for the simultaneous determination of new generation ADs and their metabolites in blood, and brain tissue was validated. In the post-mortem cases, several ADs were

quantified in different brain regions, blood and hair. While blood is the preferred matrix to link concentration and effect, analysis of brain tissue and hair can be of interest to investigate decayed corpses, or to increase the detection window.

Keywords: post-mortem brain tissue, antidepressants, metabolites

P105. Simultaneous extraction of amphetamine, buprenorphine, cocaine, methadone, methylenedioxymethamphetamine, morphine and zolpidem from hair analyzed by LC-MS/MS

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Objective: A method by Kronstrand et al. [Forensic Sci Int. 2004; 145: 183-90] for simultaneous extraction of drugs from hair has been tested, expanded and then validated for seven drugs: amphetamine (AMP), buprenorphine (BUP), cocaine (COC), methadone (MET), methylenedioxymethamphetamine (MDMA), morphine (MOR) and zolpidem (ZOL). These seven drugs have been chosen for their differences in physically and chemically properties. Development of a simple and versatile method for detection of these different drugs in hair has been favoured, opening the possibility for the method to be expanded to other drugs.

Method: The extraction procedure consists of the following steps; (1) Homogenization of hair; (2) incubation of 10 mg hair with 500 µL methanol: acetonitrile:20mM formate buffer pH 3.0 (10:10:80) and 25 µL of internal standard (3.2 µg/mL of D_5-AMP, D_4-BUP, D_3-COC, D_3-MET, D_5-MDMA and D_6-MOR) at 37°C for 18 h; (3) centrifugation followed by direct injection of 5 µL of the supernatant onto LC-MS-MS. Analysis is done by LC-MS-MS with electrospray ionisation, using a C18-column and a gradient system consisting of mobile phase A (0.05% formic acid in water:methanol (950:50)), and mobile phase B (0.05% formic acid in methanol) separating the seven drugs successfully within 23 minutes.

Results: Linearity is found for all seven drugs between 0.05 ng/mg – 50 ng/mg (R^2> 0.999 for all seven drugs). Table 1 summarizes LOD, LOQ, recovery after extraction at 5.0 ng/mg, intermediate precision (n=9 for analysis of Proficiency Test DHF 2/07 from GTFCH) and accuracy (n=9, DHF 2/07) when the method validation is performed following ICH guidelines.

Table 1. Results of method validation.

Drug	LOD (ng/mg)	LOQ (ng/mg)	Extraction recovery (%)	Intermediate precision (% CV)	Accuracy (%)
AMP	0.005	0.016	92.4	9.0	92.2
BUP	0.015	0.044	90.6	9.1 *	95.6 *
COC	0.002	0.007	92.0	15.0	78.4
MET	0.006	0.018	91.3	6.1 *	96.0 *
MDMA	0.002	0.006	91.7	10.7	95.8
MOR	0.009	0.027	92.8	8.8	90.4
ZOL	0.011	0.032	90.2	4.9 *	94.2 *

DHF 2/07 only contains AMP, COC, MDMA and MOR. Intermediate precision and accuracy for BUP, MET and ZOL is determined for blank hair spiked with these drugs (n=12, spiked in the range 0.5-25 ng/mg)

Furthermore the newest Proficiency Test, DHF 3/07, from GTFCH has been analyzed using the validated method above. Quantification of AMP, COC, MDMA and MOR was successfully performed within acceptance criteria. Also, hair spiked with a solution of different basic drugs was analyzed using the validated method, resulting in successful extraction of 18 basic drugs from hair.

Conclusion: In conclusion this method is useful for extracting AMP, BUP, COC, MET, MDMA, MOR and ZOL from hair, with a further possibility for expansion to other drugs in hair.

Keywords: hair, pharmaceuticals and drugs of abuse, LC-MS/MS

P106. Screening of amphetamines in hair using a homogenous immunoassay procedure

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Introduction: Hair is a useful specimen to detect long-term use of drugs. Drug concentrations are usually low and sample volume is often limited, so sensitive screening methods are necessary. Following methamphetamine (METH) use, METH itself is generally found in hair, but amphetamine (AMP) may be present as a metabolite, or as an independent drug.

Methods: Hair (10 mg) from METH users (n=20) as well as hair from drug free volunteers (n=20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL) and incubated (3 hrs/75°C). The supernatant was analyzed using two enzyme linked immunosorbent assays (ELISA) and two homogeneous immunoassays (HEIA) on an Olympus 400 platform, one for METH, one for AMP. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for HEIA, 10 µL was used, making the process compatible with most commercial chemistry analyzers.

Results: A screening cutoff of 500 pg/mg was used. The intra-assay precision at 250, 500, 1000 and 2500 pg/mg was determined to be 9.9%, 8.2%, 6.6% and 3.7% for AMP; 11.6%, 8.3%, 7.1% and 2.7% for METH respectively. All the negative specimens screened negatively using both ELISA and EIA. The results of the positive specimens are shown below.

Sample	ELISA		HEIA Cutoff 500pg/mg		GC/MS (pg/mg)	
	AMP	*METH*	*AMP*	*METH*	*AMP*	*METH*
1	P	P	P	P	930	>10,000
2	P	P	P	P	103	6121
3	P	P	P	P	283	2360
4	P	P	**N**	P	257	8671
5	**N**	P	**N**	P	233	9273
6	N	P	**N**	P	105	2046
7	P	P	**N**	P	2883	>10,000
8	P	P	P	P	311	7953
9	P	P	**N**	P	219	2707
10	P	P	P	P	108	6665
11	P	P	P	P	2390	>10,000
12	P	P	P	P	5944	>10,000
13	P	P	P	P	1089	9850
14	P	P	P	P	1825	>10,000
15	P	P	P	P	3689	>10,000
16	P	P	P	P	1130	>10,000
17	P	P	P	P	1135	>10,000
18	P	P	P	P	1115	9512
19	P	P	P	P	775	7088
20	P	P	P	P	645	5560

Cross-reactivity: The METH antibody cross-reacts with MDMA (65%); the AMP antibody cross-reacts with MDA (45%). The assay is precise, sensitive and conducive to rapid hair screening using commercial chemistry analyzers.

Keywords: homogeneous immunoassay; amphetamines; hair

P107. Qualitative screening of cocaine in hair using a homogenous immunoassay procedure

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Introduction: Hair is a useful specimen to detect long-term use of drugs. Hair generally contains low drug concentrations and sample volume is often limited. Following cocaine use, the main drug detected in hair is the parent drug itself, so a screening immunoassay should be targeted to cocaine.

Methods: Hair (10 mg) from cocaine users (n=19) as well as hair from drug free volunteers (n=20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL), and the hair was incubated (3 hrs/75°C). The supernatant was analyzed using enzyme linked immunosorbent assay (ELISA) and by homogeneous immunoassay (HEIA) on an Olympus 400 platform. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for homogenous EIA, 20 µL was used directly, making it conducive to commercial chemistry analyzers.

Results: An HEIA targeted at cocaine using a screening cutoff of 500 pg/mg has been developed. The intra-assay precision at 250, 500, 1000 and 2500 pg/mg of cocaine was determined to be 7.1%, 12%, 9.3% and 3.8% respectively. All the negative specimens screened negatively using ELISA and EIA. The results of the positive specimens are shown.

Sample	**ELISA**	**HEIA**		**GC/MS results (pg/mg)**		
Cut-off	*500 pg/mg cocaine*	*500 pg/mg cocaine*	**BZE**	**Cocaine**	**NC**	**CE**
1	P	P	3492	>10,000	2746	ND
2	P	P	9531	>10,000	2419	ND
3	P	P	375	4501	62	389
4	P	P	9614	>10,000	2847	9521
5	P	P	>10,000	>10,000	4797	>10,000
6	P	P	5779	>10,000	662	ND
7	P	P	3978	>10,000	ND	8282
8	P	P	2492	>10,000	644	1589
9	P	P	6564	>10,000	722	840
10	P	P	>10,000	>10,000	3556	9868
11	P	P	7672	>10,000	2347	ND
12	P	P	>10,000	>10,000	2287	756
13	P	P	>10,000	>10,000	7927	214
14	P	P	132	1181	ND	ND
15	**N**	P	231	728	ND	ND
16	P	P	>10,000	>10,000	ND	7113
17	P	P	>10,000	>10,000	ND	833
18	P	P	>10,000	>10,000	ND	ND
19	**N**	P	107	573	ND	ND

Cross-reactivity: The cocaine antibody cross-reacts 100% with CE, but has lower cross-reactivity to BZE. The assay is precise, specific and sensitive, and is suitable for the rapid screening of hair specimens at a cut-off concentration of 500 pg/mg of cocaine.

Keywords: homogeneous immunoassay; cocaine; hair

P108. Development and validation of a single LC-MS/MS assay following SPE for simultaneous hair analysis of amphetamines, opiates, cocaine and metabolites

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Introduction: The two major problems in hair analysis are the sample size and low targeted concentrations. To overcome these limitations, a liquid chromatography-electrospray-tandem mass spectrometry method (LC-MS/MS) allowing the simultaneous analysis of 27 amphetamines, opiates, cocaine and metabolites has been developed.

Method: This LC-MS/MS method allows the identification and quantification of 17 amphetamines (amphetamine*, BDB, m-CPP, dexfenfluramine, DOB, DOM, ephedrine*, MBDB, MDA*, MDEA*, MDMA*, methamphetamine*, methylphenidate, 4-MTA, norephedrine, norfenfluramine and PMA), 4 opiates (morphine*, codeine*, ethylmorphine, 6-MAM*), cocaine* and 5 metabolites (ecgoninemethylester (EME)*, benzoylecgonine (BZE)*, anhydroecgoninemethylester (AE), cocaethylene*, norcocaine*). After decontamination and cutting steps, internal standards (*deuterated equivalents) were added to 50 mg of hair sample. Analytes were extracted for 18 h at 45°C using phosphate buffer (pH 5), followed by SPE clean-up using MCX® extraction cartridges (Oasis®, Waters). Analytes were separated on an Atlantis T3 column (Waters, 150 mm x 2.1 mm, 3 µm) by a gradient of acetonitrile and formate buffer (2 mM, pH 3) and detected in Multiple Reaction Monitoring mode (API 2000, Applied Biosystems®). For each analyte, two transitions were monitored: one for the quantification and one for the confirmation. Our validation procedure consisted of the study of linearity, intra-day and inter-day variability and accuracy for 5 days (5 replicates at 3 concentration levels). External quality controls were also analysed to check the accuracy of the method. Using this whole procedure, the method was routinely applied to hair samples.

Results: 17 amphetamines, 4 opiates and 6 cocaine derivatives are satisfactory identified by MRM in 14 minutes. Calibration curves are performed either by "linear through zero" or "quadratic 1/X weighting" regression. The method is linear from 0.05 to 10 ng/mg. The limits of detection (lod) range between 0.001 and 0.02 ng/mg. Precision has been checked by intra-day and inter-day CVs and associated relative bias, which are lower than 25%, 21% and 22%, respectively. The results of 3 GTFCh's external quality controls (spiked and drug addicts' hair) confirm the accuracy of the method. The following table presents 3 positive results of adult drug addicts (patient A: global analysis; patients B and C: segmental analysis; nd=not detected:<lod):

		Concentration (ng/mg)									
S	Segment	EME	AE	Coc.	Norcoc.	BZE	Codeine	Morph.	6-MAM	MDA	MDMA
A	global	0.5	0.5	11.0	0.6	2.4	1.0	4.84	8.89	nd	<0.05
B	0-1.5cm	<0.05	<0.05	0.17	<0.05	<0.05	0.07	nd	nd	0.11	0.87
	1.5-3cm	<0.05	<0.05	0.15	<0.05	<0.05	<0.05	nd	<0.05	0.08	0.78
C	0-1cm	nd	nd	nd	nd	nd	<0.05	0.20	0.07	nd	nd
	1-2cm	nd	nd	nd	nd	nd	<0.05	0.15	0.13	nd	nd
	2-3cm	nd	nd	nd	nd	nd	<0.05	0.25	0.39	nd	nd
	3-4cm	nd	nd	nd	nd	nd	<0.05	0.52	0.81	nd	nd
	4cm-end	nd	nd	nd	nd	nd	0.07	0.56	1.57	nd	nd

Conclusion: This sensitive LC-MS/MS method allows the simultaneous identification and quantification of amphetamines, opiates, cocaine and metabolites in a 50mg hair sample and could be considered as useful for clinical and forensic toxicology diagnostic purposes.

Keywords: drugs of abuse, LC-MS/MS, hair

P109. Niaprazine in hair. Children under influence

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Introduction: Niaprazine, under the trade name Nopron, is largely used in France as an hypnotic agent for occasional insomnia of children. This compound is available without medical prescription. The first case involving repetitive administration of niaprazine as a drug-facilitated sexual assault is reported. Three children (2 girls and 1 boy) were repetitively sedated and assaulted by their father-in-law between 2002 and 2006. Niaprazine's liquid formulation represents a good potential access to surreptitiously administer it in beverages. According to the request of the judge in charge of this case,

hairs of victims were collected, segmented and screened for sedatives by LC-MS/MS.
Method: After decontamination and cutting in small pieces, 20 mg of hair was incubated overnight in a phosphate buffer (pH 8.4). The aqueous phase was extracted by 5 ml of a mixture of diethyl ether/methylene chloride (80/20), in presence of diazepam-d_5, used as internal standard (IS). Hair extract was separated on a XTerra MS C18 column using a gradient of acetonitrile and formate buffer. Drugs were identified by 2 transitions (m/z 357>106 and 177 and 290>154 and 198 for niaprazine and IS, respectively). LOQ of niaprazine was 10 pg/mg.
Results: Niaprazine was detected in the range 21 to 382, <LOQ to 315, 2642 to 3431 pg/mg for the three children, respectively. These concentrations could not be compared with previous results, due to a lack of literature. In particular, it was not possible to put any quantitative interpretation on the dosage that was administered to the children. It is however obvious that repetitive administrations have occurred but it is not possible to determine the number of exposures. Given the length of the hair, exposure to niaprazine should have occurred at least during the previous months.
Conclusion: The surreptitious administration of niaprazine to obtain sedation was considered as a drug-facilitated crime, even in an intra-familial situation. According to the French law, the drug can be considered as a chemical weapon.
Keywords: hair, children, niaprazine, drug-facilitated crime

P110. Determination of nicotine in hair samples of 1000-year old mummies

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Introduction: In the literature there exist some reports about cocaine, nicotine and cannabinoids found in ancient hair of mummies from South America or Egypt. Most of the results were critically discussed for the use of improper techniques or contamination of the sample material. Recently, an exhibition of 70 mummies from around the globe was organized at a German museum. The display presents exhibits assembled by the world famous mummy project, involving various specialists for anthropology, pathology, radiology, molecular biology and toxicology.
Methods: Hair samples of seven ancient Peruvian mummies were analyzed for drugs of abuse (cannabinoids, opiates, cocaine-like substances) using modern routine GC-MS techniques. Additionally a GC-MS screening procedure was performed following methanolic ultrasonication of the samples. For the analysis of nicotine and cotinine a GC/MS procedure employing deuterated internal standards was performed in selected ion monitoring mode (3 characteristic ions).
Results: The tests revealed negative results except for nicotine in the hair of 3 mummies. Nicotine was measured in concentrations of 57.5 ng/mg in the hair of a woman, 14.1 ng/mg in a child and 11.4 ng/mg in a further female mummy, but all cases revealed negative results for cotinine. The washing solutions yielded negative results for both analytes, i.e. nicotine as well as cotinine.
Discussion: In 3 out of 7 hair samples of ancient Peruvian mummies nicotine was found in concentrations which were reported in cases of active smokers. It has to be considered however that the nicotine metabolite cotinine was not detected in any case. This typical nicotine metabolite would indicate an active ingestion (body passage), due to lower concentrations the metabolite is however not even detectable in every active smoker. In our opinion, even with respect to negative results in the washing solutions, the present results cannot definitely confirm an active consumption in the life time of the analyzed mummies: an external contamination, e.g. by transfer from smoking visitors or employees of the museum as well as in their respective lifetime, cannot be excluded. Computer tomography (CT) scans revealed symptoms of tuberculosis in one female mummy. It is thus also plausible, that an external contamination of this mummy's hair resulted from a ritual use of the 'sacred' tobacco plant, e.g. during a ceremony in which a shaman would treat the patient with tobacco smoke.
Keywords: nicotine, hair analysis, mummy

P111. Detection of amitriptyline and nortriptyline in various organs and hair by LC-MS/MS in the homicidal fatality of a one-month-old girl

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Case report: A four-year-old girl was admitted to an intensive care unit in a desperate state. She died a few minutes later. Toxicological analyses revealed a *post-mortem* blood concentration of the antipsychotic drug cyamemazine at a concentration of 3,4 µg/mL, which was the direct cause of death. Informed of the facts, the prosecutor then ordered the exhumation of a one-month-old baby, deceased 6 months beforehand in circumstances which at the time had been considered normal. The body, locked in a zinc coffin, was rather well preserved. Toxicological analyses were carried out of the liver, the intracerebral liquid, and a 3-cm lock of hair.
Method: After crushing the viscera, a pre-treatment by subtistilline-A and a liquid-liquid extraction, analyses were realised by HPLC-MS/MS in positive ionisation mode on a C_{18} analytical column using a gradient of acetonitrile and 2 mM formate buffer at pH=3. The hair was pulverized using a ball mill, then sonicated for 2 hours in methanol, and subsequently purified by solid phase extraction on OASIS HLB® cartridges. Quantification is based on the main ion m/z=233 common daughter of m/z=264 for nortriptyline, m/z=278 for amitriptyline and m/z=267 for nortriptyline D3 used as internal standard.
Results: The massive presence of amitriptyline and nortriptyline in the liver was measured at a concentration of 29,8 and 3,6 µg/g. According to the scientific data, these concentrations are high enough to cause death. Hair analyses revealed the presence of amitriptyline and nortriptyline at concentrations of 1811 and 53 pg/mg respectively, while complementary analyses showed the presence of bromazepam in the hair at a concentration of 740 pg/mg, thus documenting previous administrations. The mother would confess later to having used the drinkable form of the pharmaceutical LAROXYL® by pouring the content of a 20 mL bottle (at 40 mg/mL) into the feeding-bottle of her child. The milk was sweet but still bitter and the whole family helped to feed the crying baby.
Conclusions: We reported on an unusual homicide by poisoning. This method is the first to describe LC-MS/MS analysis of amitriptyline and nortriptyline in hair.

Keywords: amitriptyline, death, hair

P112. Analysis of carbofuran and 3-hydroxycarbofuran in hair: interpretation difficulties

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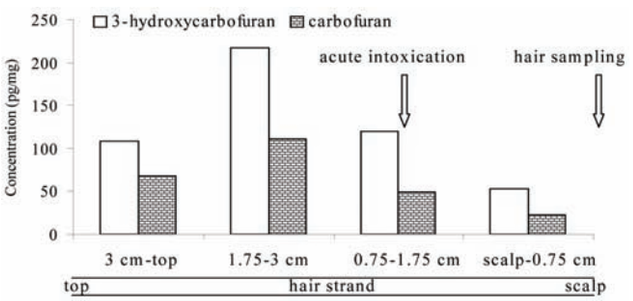
Introduction: Carbofuran is a carbamate insecticide used for the treatment of soil, exhibiting systemic properties known to be highly hazardous for humans. We report here two cases of non fatal intoxication with carbofuran for which concentration values in hair have been measured. The first patient history presented numerous acute intoxication episodes (attempted homicide) and the second patient had only had one exposition (a single attempted suicide).

Methods: Detection and quantitation of carbofuran and its metabolite, 3-hydroxycarbofuran, were performed using a Sorensen buffer incubation followed by a liquid-liquid extraction and a liquid chromatography-electrospray-tandem mass spectrometry (LC-ES-MS/MS) analysis according to the method described by Dulaurent et al. [Forensic Sci Int. In Press].

Results: For the first patient, three hair strands were sampled at three different periods at 1.5 and 8.5 months after the first sampling. Carbofuran and 3-hydroxycarbofuran were found in all segments of the three strands with concentrations ranging from 23 to 557 pg/mg and 50 to 645 pg/mg respectively. Nevertheless, in the last hair strand (6.5 cm length) sampled 20 days after the last exposure, carbofuran and its metabolite were found in all segments whereas the previous acute intoxication had taken place 8.5 months before. For the second patient, a single hair strand was sampled 1.5 month after the single contact. The following figure presents concentrations profile of his hair strand:



Conclusion: Carbofuran and its metabolite could be detected in hair of victims of acute intoxication. Nevertheless, we found also these products in hair segments, which correspond to a period before exposure. What could be the explanation(s): a) migration of these compounds in the hair? b) sweating effect (i.e.: when a carbamate intoxication occurs, there is an enhance of sweating which can contaminate the whole length of the hair)? c) accumulation of compounds in lipid tissues during previous contacts and spreading of these compounds from lipid tissues to hair by way of blood? d) pollution of all segments of the hair strand during dichloromethane decontamination?

Keywords: pesticide, hair, LC-MS/MS

P113 Characterization of addictives practices by a combining general unknown screening and targeted screening by UPLC-MS/MS in hair: A case report

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Introduction: A 24-year old man, known for abuse of drugs, was arrested for torture and acts of cruelty against his 4-year old child. Two weeks later, the judge orders a hair analysis of father. His hair was cut from the root (3 cm) in order to estimate his full drug use history during the last three months.

Materials and Methods: The hair sample was twice decontaminated using 5 ml methylene chloride and cut into small pieces (≈1mm). About 85 mg were incubated overnight in phosphate buffer at pH 8.4, in the presence of a internal standard. After liquid – liquid extraction with a mixture of methylene chloride/diethyl ether (90/10) and evaporation to dryness, the residue was reconstituted in 100 µL of mobile phase. 15 µL were injected into the ACQUITY UPLC-MS/MS system (Waters). Separation was achieved on ACQUITY UPLC™ HSS C18, 1.8 µm (2.1 x 150 mm). Mobile phase (ammonium formate buffer 5 mM pH=3/ACN) was delivered in gradient mode for a total run time of 15 min. The detection was performed by ACQUITY TQ Detector (Waters) in single MS mode and MRM mode. Single MS scan mode from 80 to 650 m/z in positive ESI (4 values of cone voltage) and in negative ESI (2 values of cone voltage). The MRM mode used 2 transitions and their ratio to confirm the presence of each compound. Each molecule was matched against a MS library of 527 compounds and a MRM library of 138 compounds.

Results: After 2 screenings, several drugs were matched:

	UPLC/MS/MS	UPLC/MS
Codeine	+	+
Morphine	+	+
6 MAM	+	+
MDA	+	
MDMA	+	
Amphetamine	+	
Benzoylecgonine	+	+
Cocaine	+	+
Cocaethylene	+	
Paracetamol	+	
Hydroxyzine	+	+
Cetirizine	+	
Propoxyphene	+	+
Cyamemazine	+	
Meprobamate	+	
Diazepam	+	
Papaverine		+
Noscapine		+
Phenacetine		+
Pheniramine		+
Nicotine		+
Cotinine		+

Conclusion: Once more, this case demonstrates that hair analysis can be used to detect drugs which were taken several months ago. The power of Acquity UPLC-MS/(MS) contributes widely to this result.

Keywords: hair analysis, drug use history, UPLC-MS/MS

P114. Detection of nalbuphine in postmortem samples

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Aims: A clinical investigation on a double-blind crossover study of 17 volunteers who received an intravenous injection of nalbuphine (5 mg or 10 mg), morphine (5 mg) or placebo. In these respects, the potency ratio of nalbuphine appears roughly equivalent to morphine. In order to evaluate the nalbuphine concentrations after chronic nalbuphine administration a rat model was used to predict the findings in human tissues. Identification and detection of nalbuphine is very important as it gives us a great help in revealing the mystery of deaths resulting from taking overdoses of nalbuphine. If we can identify and detect nalbuphine in different tissues and body fluids, and also specification of the tissues which have the highest and lowest concentration of nalbuphine, will help us in deciding which organ will be the best postmortem sample that have the highest concentration of nalbuphine.

Methods: Ten male albino rats were divided into two groups, each group consisting of five rats. The first group was given distilled water only and used as controls. The second group treated with nalbuphine at LD_{50} (297mg/kg) i.v. [Mier J Toxicon. 1986; 124: 395-401].

The rats were sacrificed after three hours and dissected. Specimens collected included liver, kidney, brain, heart blood and hair. Nalbuphine was extracted from tissues and blood by liquid - liquid extraction using ammonium sulphate and methylene chloride. Hair samples were washed in deionized water for 5 min to eliminate traces of blood. This was followed by 3 brief of rinses in methanol to remove any other surface contaminations. Hair

samples were subsequently dried and weighed. The hair was dissolved with sodium hydroxide, then hydrolysed with concentrated hydrochloric acid until pH 9 was obtained and extracted for nalbuphine by methylene chloride. Nalbuphine was quantitated by HPLC-UV using a C18 column, methanol as a mobile phase, detection at λ=254 nm [Couper F.J et al. J Forensic Sci. 1995; 40(1): 87-90]. Chromatogram from control rats were used to compare HPLC chromatogram of treated rats. Nalbuphine metabolites were not evaluated in this study.

Results: The distribution of nalbuphine in treated rats in summarized in the table below:

Table 1. Distribution of nalbuphine (mg/g) in different organs after a dose of 297 mg/kg.

Animal No.	Blood	Brain	Hair	Kidney	Liver
1	8.65	67.88	52.22	40.40	5.52
2	7.88	66.00	51.11	39.18	4.98
3	9.02	68.13	53.02	41.00	6.04
4	8.80	66.98	52.19	40.60	5.70
5	8.70	67.12	54.00	42.01	5.30
average	8.61	67.22	52.51	40.63	5.51

Table 2. Total abundance of nalbuphine in different organs after a dose of 297mg/kg.

Drug	1	2	3	4	5
Nalbuphine	Brain	Hair	Kidney	Blood	Liver

Calibration graphs of nalbuphine in blood sample were linear from 0.2-10 mg/L. Detection limit was 0.1 mg/L and quantitation limit was 0.2 mg/L. Calibration graphs of nalbuphine in brain sample were linear from 0.005-0.017 mg/L. Detection limit was 0.005 mg/L and quantitation limit was 0.004 mg/L.

Calibration graphs of nalbuphine in hair sample were linear from 0.01-10 mg/L. Detection limit and quantitation limit were 0.01 mg/L. Calibration graphs of nalbuphine in kidney sample were linear from 10-20 mg/L. Detection limit was 0.1 mg/L and quantitation limit was 0.2 mg/L. Calibration graphs of nalbuphine in liver sample were linear from 0.3-5 mg/L. Detection limit and quantitation limit were 0.3 mg/L. The unit for the hair concentration was (mg. conc/g, organ). All organs were extracted at pH=9. The present study would be of great importance of investigation of any overdose toxicological mystery death of nalbuphine, and we conclude that the best organ of postmortem sampling as it has the highest concentration of nalbuphine (brain, hair, and kidney) and can help us in extracting nalbuphine to be identified and detected.

Conclusions: From the present study we conclude that nalbuphine toxicity could be predicted in various tissues, but it exhibits the highest concentration in brain, hair and kidney. The highest concentration of nalbuphine was detected in brain since the brain receives one-sixth of the total amount of blood leaving the heart. Lipid soluble drugs are distributed to brain tissue very rapidly compared with other tissues. Hair has the next highest nalbuphine concentration since nalbuphine moves by passive diffusion from the blood stream into the hair cells at the base of the follicle and are then bound in the interior of the hair shaft. Kidney concentrations are the next highest since there is little nalbuphine excreted.

Keywords: nalbuphine, tissue distribution, toxicity

P115. A liquid-chromatography-electrospray ionization-tandem mass spectrometry method following SPE extraction dedicated to the determination of ethyl-glucuronide in hair

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Introduction: Ethyl-β-D-6-glucuronide (EtG) is a stable Phase II metabolite of ethanol. Its detection in hair is of interest in both a clinical and forensic context with the aim of monitoring alcohol abuse. We present a validated liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method dedicated to EtG analysis in hair.

Methods: To 30 mg of a washed and cut (1-2 mm length) hair sample, 2 mL of water and 100 µL of internal standard solution (EtG-D5, 100 µg/L in methanol) were added. After ultrasonication (2h, 50°C), the extracts were cleaned-up with graphite SPE cartridges (Clean Screen, UCT, USA). Chromatography was then performed using an Uptisphere-3SI Column, 100 x 2 mm, 3 µm particle size (Interchim, France) and the mass spectrometer was operated in the negative ion mode for detection. The MRM transitions monitored were: m/z 221→ 75 and 221→ 85 for EtG, 226→ 75 for EtG-D5. Validation procedure was performed according to the international proposed experiments, evaluations, and acceptance criteria for validation of new analytical methods in this context. The intra- and inter-assay precision and accuracy were assessed at four concentration levels relative to calibration range. In addition, the developed method was applied to several hair samples taken from: 4 fatalities (F) with documented excessive alcohol consumption, 12 known alcoholics (HD: heavy drinkers) according to physicians of the emergency unit and 7 social drinkers (SD).

Results: The method exhibits a detection limit of 4 pg/mg, a quantification limit of 10 pg/mg and the calibration curves were linear from 10 to 3000 pg/mg. Intra- and inter assay precision standard deviation and relative bias were less than 20% over the calibrating range. In addition, no influence of interfering compounds on the signal was observed for both EtG and EtG-D5. For all SD patients EtG hair concentrations were <10 pg/mg and for every HD as well as F patients, EtG hair concentrations were >50 pg/mg. The following table presents the results for HD patients:

	heavy drinkers (n=12)											
Blood ethanola	3.8	<0.1	3.2	0.4	3.9	3.6	5.1	<0.1	1.9	3.0	4.9	4.6
MCVb	98	99	106	102	107	90	95	94	83	97	99	100
GGTc	444	509	140	185	341	46	422	51	57	104	42	40
EtGd	180	54	370	341	303	60	252	60	92	66	497	365

[a]g/L at time of hair sampling; [b]Mean corpuscular volume in fL;
[c]gamma-glutamyltransferase (U/L); [d]pg/mg of hair

Conclusion: This method is fully validated. The choice of the cut-off value remains a real problem as values between 30 and 100 pg/mg can be found in the literature. Our preliminary results suggest that a EtG concentration ≥50 pg/mg can be related to an effectively heavy alcohol consumption. A lower cut-off value (30 pg/mg?) could perhaps be proposed. However, in order to avoid the risk of false positive results due to the high sensitivity of such a LC-MS/MS method, further studies are needed to clarify cut-off values of EtG concentrations in hair for diagnostic purposes.

Keywords: hair, ethyl-glucuronide, LC-MS/MS

P116. Clinical symptoms and degree of cholinesterase inhibition are depending on organophosphate compounds

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Objective: Through the world, organophosphate (OP) compounds are used as insecticides and induced hundreds of thousand cases of poisonings. Although, the treatment of OP poisoning does not dependent on the structure of the organophosphates. Different studies reported evidence that the toxicity of OPs is different between the compounds. To test this hypothesis, we determined the degree of inhibition of total cholinesterase in whole blood and tissues of rats poisoned by different organophosphate or carbamate insecticides at the LD_{50} doses determined in your laboratory.

Methods: Five organophosphates (paraoxon, chrlorpyrifos, dichlorvos, dimethoate, and fenthion) and one carbamate (methomyl) were studied. The LD_{50} were determined using the "up and down" method of Bruce and Dixon. Animals were poisoned by SC (paraoxon, dichlorvos), IP (methomyl), and oral (chlorpyrifos, fenthion, dimethoate) routes using three doses (0.5 LD_{50}, LD_{50} and 2 LD_{50}). Animals were observed until the death or a maximal long period of time of 7 consecutives days and clinical symptoms and time of death were recorded. The experimental LD_{50} were determined as the median value of the three doses. Secondly, rats were poisoned by each insectide at the experimental LD_{50} and sacrified at the climax of the clinical symptoms using a dioxide gas chamber. Whole blood specimens were sampled and immediately diluted (1/20) in distilled water. Rats were washed-out from blood using saline isotonic perfusion by cannulation of aorta. Tissues (diaphragm, lungs, brainstem, thigh muscle, brain frontal area) were immediately taken. All samples were kept to –80°C until the time of determinations. At the time of dosage, tissues (about 200 mg) were crushed in isotonic saline with Triton X100 (1% v/v), then centrifuged. Total cholinesterase activities were measured by radiometric assay in diluted whole blood and in tissue supernatants. Cholinesterase activities were expressed as $UI.g^{-1}$ of hemoglobin for whole blood and in $UI.g^{-1}$ of proteins for tissues. Final results (mean ± SEM) are expressed as per cent of residual activities compared to control group. Statistical analysis was performed using ANOVA tests with $p<0.05$.

Results: Experimental LD_{50} were 0.48 (paraoxon), 4.6 (methomyl), 12.8 (dichlorvos), 218 (chlorpyrifos), 247 (dimethoate), and 404 (fenthion) $mg.Kg^{-1}$, similar to the values previously reported in the literature. For paraoxon, dichlorvos and methomyl, mean times of death was below 30 min and fasciculations were the major clinical symptom observed between 5 (methomyl) to 15 min (dichlorvos) after insecticides administration. For others compounds, mean times of death ranged from 24 to 40 hours with ataxia as major clinical symptom. The delayed toxicity can be explained by the metabolic activation of these compounds (from thion to oxon). Dimethoate, fenthion, and methomyl were more potent inhibitor than paraoxon considered as the most toxic OP insecticides. Dimethoate and fenthion induced a complete inhibition in diaphragm and thigh muscles. No apparent relation was observed between the lipophylic property of the compound and the degree of inhibition of cholinesterase activities in fatty organs including brain frontal area or brainstem.

Conclusion: This study in rats evidenced that insecticides compounds have different toxicities regarding the clinical signs and symptoms induced by LD_{50}, the times of death as well as the degrees of cholinesterase inhibition.

Keywords: organophosphates, cholinesterase, clinical symptoms

P117. Pharmacokinetics of diamorphine in children following intravenous and intranasal administration

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Aims: In recent years, intra-nasal diamorphine (DIM) has been recommended as an alternative to intravenous administration in children for the treatment of acute, severe pain because it is a less traumatic means of administering rapid, powerful analgesia to children in whom obtaining intravenous access may be technically difficult and distressing. This work was aimed at obtaining pharmacokinetic data for DIM and its metabolites in children following intravenous (IV-DIM) and intranasal (IN-DIM) administration in a blind study. Since DIM is known to act as a pro-drug and to achieve analgesia via its metabolites, it was intended that the concentrations of active DIM metabolites would be used to evaluate whether or not IN-DIM can deliver rapid and efficient analgesia in children comparable to that obtained with IV-DIM.

Methods: Plasma samples were obtained from twenty three children receiving DIM at the A & E department of a city-centre paediatric teaching hospital in Edinburgh. 13 children received IV-DIM (dose 0.1mg/kg) and 10 subsequent children had IN-DIM at the same dose in 0.2 ml normal saline dripped into both nostrils. The children were aged 3-13 years, with clinical diagnosis of isolated deforming limb fractures. Sequential blood samples were taken at 2, 5, 10, 20, 30 and 60 minutes post DIM administration. The blood tube was then centrifuged at 4000 rpm for 2 minutes and plasma was transferred to plain 'Ependorph' tubes that were immediately placed in a -70oC freezer until analysed. Plasma samples were subsequently analysed for DIM, 6-monoacetyl morphine (6-MAM), morphine (MOR), morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G) and normorphine (NMOR) using a method involving solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS-MS) that was validated for this purpose.

Results: Concentrations of analytes were found to depend on the route of administration. The concentration of DIM metabolites using IN-DIM were lower than IV-DIM at the same dose and body weight. DIM concentrations were in the range 190 to 2062 ng/mL and 2 to 43 ng/mL for IV-DIM and IN-DIM, respectively. The median peak concentrations of analytes of interest after IV-DIM were achieved at 2 min for DIM, 6-MAM and MOR, and at 20 and 60 min for M3G and M6G respectively. After IN-DIM, median peak concentrations were achieved for DIM at 2 min. However, DIM metabolites achieved different median peak concentrations after IN-DIM: at 5 and again at 10 min for 6-MAM, at 20 min for MOR and at 60 min for both morphine glucuronides. Ratios of concentrations (IV-DIM/IN-DIM) were calculated using the median peak concentrations and were 34, 26.4, 6.2, 3.8 and 2.9 for DIM, 6-MAM, MOR, M3G and M6G respectively. Following IV-DIM, the concentrations of DIM, 6-MAM and MOR decreased sharply after 2 min whereas the rate of decrease was slower after IN-DIM. Pharmacokinetic parameters i.e. Cmax, Tmax, t½ and the area under the plasma curve were calculated for the two patient groups. These were compared with each other and with previous work in adult patients.

Conclusions: The pharmacokinetics of DIM and its metabolites following IN and IV administration in children have been compared for the first time in this study, which confirmed that IN-DIM can achieve therapeutic plasma concentrations of active metabolites, although these are lower than those obtained with IV-DIM and occur at later times after administration.

Keywords: diamorphine metabolites, children, intravenous, intranasal

P118. Concentration of fentanyl in blood after administration of transdermal patches in normal weight and cachectic patients with cancer related pain

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Introduction: Fentanyl is a synthetic μ-opioid receptor agonist used in the treatment of acute and chronic pain. The high lipid solubility and low molecular weight make fentanyl suitable for transdermal administration. The strong potency provides usage of low doses, requiring a very sensitive analytical method for determination of fentanyl in blood. In clinical practice, cachectic cancer patients seem to require higher transdermal fentanyl doses for adequate analgesia than normal weight or obese cancer patients. The objectives of the study were to develop an LC-MS/MS method for determination of fentanyl in human blood, and to investigate differences in blood concentration of fentanyl in cachectic and normal weight patients with cancer related pain.

Methods: Blood samples were extracted with butyl acetate at pH 7. The extracts were run by Agilent LC 1100 binary pump system on a Genesis C18 reversed phase column. The mass spectrometric analysis was performed with an AB/MDS Sciex 3200 QTrap LC-MS/MS instrument using multiple reaction monitoring (MRM). The collision energy and declustering potential were 30 eV and 40 V, respectively. The monitored transitions were m/z 337/188 and m/z 337/105 for fentanyl, and m/z 342/188 for fentanyl-d5. Quantitation was based on a 6-point calibration from 0.02 μg/L to 200 μg/L using fentanyl-d5 as an internal standard. Twenty-four patients with cancer related pain were recruited: fourteen normal weight (BMI 20-25 kg/m²) and ten cachectic (BMI<18 kg/m²) patients. The transdermal fentanyl patch was applied to the skin of the upper arm on Day 1. The dose of transdermal fentanyl was not increased during the study, and the initial delivery rate of fentanyl was 25-300 μg/h. Breakthrough pain was treated as needed using oral immediate-release oxycodone solution. Blood samples for determination fentanyl concentration were drawn at baseline, 4h, 24h, 48h, and 72h (±1h).

Results: The developed analytical method for fentanyl was linear from 0.08 to 530 μg/L (R2=0.992). The average intra-assay precision, inaccuracy, and LOQ were 10%, 17%, and 0.08 μg/L in blood, respectively. The pharmacokinetic data of ten normal weight (mean BMI 23 kg/m²) and ten cachectic (mean BMI 16 kg/m²) patients was analyzed. The total amount of absorbed fentanyl versus time did not differ between cachectic and normal weight patients. Top blood concentration was achieved between 24-48 hours after administration of the patch and it varied between 0.2 and 5.4 μg/L. Median time to top concentration was 36 h in the cachectic group and 24 h in normal weight patents.

Conclusion: The developed LC–MS/MS method was sensitive enough for determination of fentanyl in blood at low concentrations. Transdermal fentanyl was equally well absorbed in cachectic and normal weight patients with cancer related pain, whereas time to maximum blood concentration was longer in the cachectic group.

Keywords: transdermal fentanyl, pharmacokinetics, LC–MS/MS

P119. Carbohydrate-deficient transferrin (CDT) as biomarker for forensic diagnosis of chronic alcohol abuse in drivers.

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Introduction: The chronic alcohol abuse diagnosis, when applied in drivers sanctioned for driving under alcohol effects, represents a strong forensic evaluation which must be supported by reliable inquiries. This diagnosis can't even find a complete support in alcohol blood rate or in breath test, that are useful only to check the recent alcohol abuse. So that Carbohydrate-deficient transferrin (CDT) affirmed as a biomarker of chronic moderate to heavy alcohol consumption (more than 40-60 g ethanol/die). The main advantage of CDT is its high specificity when compared with others conventional markers, like gamma-glutamyltransferase (GGT) and mean corpuscular volume (MCV). Furthermore CDT underlines alcoholic beverages abuse in a time ranged from 2-3 weeks before a blood sampling [Appenzeller BM et al. Drug Alcohol Depend. 2005; 79(2): 261-265; Golka K et al. J Toxicol Environ Health B Crit Rev. 2004; 7(4): 319-337; Schellenberg F et al. Alcohol Alcoholism. 2005; 40(6): 531-534].

Materials and methods: This report shows data obtained from different subgroups of drivers (n. 1187) for which toxicological controls were requested in order to reapplication of the licence after driving under alcohol influence. For each subject, determination of CDT % as been performed by HPLC commercial method (BIO-RAD) on 3 serum samples, collected at random in a 30 days period, without notice. Since 2006, VEQ proposed by GTFCH (Germany) for CDT % was carried out with adequate results on our laboratory.

Results: For the evaluation of toxicological results of the DisialoTf (DST%) measured as percentage of TetrasialoTf, were adopted these different criteria: DST<1,78%=true negative; DST range 1,78- 2%=suspected for alcohol abuse; DST >2%=true positive for alcohol abuse. The results underlined, as chronic drinkers, the 9% of drivers. Among these CDT positives about 4% are drivers working in transportation activities (taxi, truck, train, bus etc.). Furthermore 4% of controlled subject (with DST ranged from 1,78- 2%) where classified as "suspected for alcohol abuse" and subjected at further controls, for three times, every six month.

Conclusion: The study confirms that CDT% can represent a marker in forensic medicine, useful for differential diagnosis both for cases of occasional or chronic heavy alcohol consumption or relapses after withdrawal, and for patients with congenital disorders of glycosylation or others enzymatic disorders not alcohol related.

Keywords: carbohydrate-deficient transferrin (CDT), alcohol abuse in drivers, HPLC