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Current awareness in drug testing and analysis

1 Reviews

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Annu Rev Anal Chem 2009 **2** 297
Forensic chemistry
A unique aspect of forensic chemistry is that it is required to meet the prerequisites of both the scientific and the legal communities in terms its research, practice, and presentation. Therefore, forensic chemistry research is applied and derivative by nature and design, and it emphasizes metrology (the science of measurement) and validation. Forensic chemistry has developed from its analytical origins and is incorporating a greater selection of chemical sciences. Existing forensic practices are being examined again as the dimensions of forensic chemistry extend from drug analysis and toxicology into such diverse areas as combustion chemistry, materials science, and pattern evidence

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Annu Rev Anal Chem 2009 **2** 485
The analytical chemistry of drug monitoring in athletes
Of importance to athletes are measures aimed at maintaining a level playing field and to decreasing risks to their health. In this respect, the detection and deterrence of the abuse of performance-enhancing drugs is paramount. The World Anti-Doping Program comprises six documents, three of which play a role in analytical development: The World Anti-Doping Code, The List of Prohibited Substances and Methods, and The International Standard for Laboratories. Amongst the classes of prohibited substances, three have resulted in the most recent analytical developments in the field: anabolic agents; peptide and protein hormones; and methods to increase oxygen delivery to the tissues, including recombinant erythropoietin. Liquid chromatography/tandem mass spectrometry and gas chromatography/combustion/isotope-ratio mass spectrometry techniques have improved the detection of anabolic agents, including designer steroids. Advances in liquid chromatography/tandem mass spectrometry has been of benefit to protein and peptide identification and quantification. The detection of blood doping has been enhanced by techniques such as flow cytometry and isoelectric focusing

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Appl Spectrosc Rev 2009 **44** (4) 317
Applications of prompt ray neutron activation analysis: Detection of illicit materials
A wide variety of materials such as metals, coal (mineral), cement, and radioactive materials as well as for explosives, chemical warfare agents, various narcotics, land mines may be analysed by prompt γ ray neutron activation analysis (PGNAA), an efficient nondestructive multi-elemental detection technique. The method may be used both in the laboratory or for on-site analysis. When combined with image scanning and chemometric treatment of the obtained data, PGNAA elemental analysis facilitates more accurate detection results with low false alerts in variety of application fields. A stationary nuclear reactor is no longer an indispensible element in PGNAA because of the development of small-sized neutron generators and thus it has enabled and widened its applications

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Annu Rev Anal Chem 2009 **2** 321
Role of analytical chemistry in defense strategies against chemical and biological attack
Two strategies of defense against chemical or biological weapons are afforded by analytical chemistry, namely, detect-to-protect and prevent-and-detect. The detect-to-protect method is based on detection of a known chemical agent with a specific chemical sensor designed for it. It is suggested that this approach has serious shortcomings. It is proposed that detect-to-protect should be replaced with the prevent-and-detect strategy. However, this change in defense strategy would require reallocation of resources. This would be required for effective protection of enclosed personel from chemical and/or biological attack

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Curr Med Chem 2009 **16** (10) 1236
Erythropoiesis stimulating agents and techniques: A challenge for doping analysts
Recombinant human erythropoietin (rHuEPO) (epoetin α and epoetin β) and its hyperglycosylated analogue Darbepoetin α are known to be misused by athletes. "EPO" is a mixture of isoforms and the *N*-glycans of the recombinant products differ from those of the endogenous hormone. Therefore, rHuEPO may be detected by isoelectric focusing (IEF) and immunoblotting of urine samples. Unfortunately, there is a abundance of novel erythropoiesis stimulating agents (ESAs). The original epoetins α and β no longer benefit from patent protection in the European Union and thus rHuEPO biosimilars have entered the market. Furthermore, several companies in Asia, Africa and Latin America produce rHuEPOs copies for clinical purposes. The amino acid sequence of all epoetins is identical. However, the structure of their glycans differs depending on the method of production. Some products include more acidic and

In order to keep subscribers up-to-date with the latest developments in their field, John Wiley & Sons are providing a current awareness service in each issue of the journal. The bibliography contains newly published material in the field of drug testing and analysis. Each bibliography is divided into 18 sections: 1 Reviews; 2 Sports Doping - General; 3 Steroids; 4 Peptides; 5 Diuretics; 6 CNS Agents; 7 Equine; 8 Recreational Drugs - General; 9 Stimulants; 10 Hallucinogens; 11 Narcotics; 12 Forensics; 13 Alcohol; 14 Tobacco; 15 Homeland Security; 16 Workplace; 17 Product Authenticity; 18 Techniques. Within each section, articles are listed in alphabetical order with respect to author. If, in the preceding period, no publications are located relevant to any one of these headings, that section will be omitted.

others more basic EPO isoforms. Epoetin δ is different because it was engineered by homologous recombination in human fibrosarcoma cells (HT-1080) and lacks *N*-glycolylneuraminic acid of native human EPO. ESAs currently being developed include EPO fusion proteins, synthetic erythropoiesis stimulating protein (SEP) and peptidic (Hematide™, CNTO 528) as well as non-peptidic EPO mimetics. Furthermore, preclinical clinical trials have been conducted with small orally active drugs that promote endogenous EPO production by activating the EPO promoter ("GATA-inhibitors": diazepane derivatives) or enhancer ("HIF-stabilizers": 2-oxoglutarate analogues). In addition, in the future, prohibited direct EPO gene transfer has the potential to become a problem

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Clin Chem 2009 **55** (6) 1061

Steroid hormone analysis by tandem mass spectrometry

Amongst the most successful methods to improve specificity problems inherent in many immunoassays are the new high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) techniques. Problems with immunoassays for the measurement of steroids are highlighted and the emerging role of LC-MS/MS in the analysis of clinically relevant steroids is reviewed. The latest generation of tandem mass spectrometers has far greater limits of quantification, facilitating omission of previously employed derivatization steps. Steroid analysis is necessary for the diagnosis and treatment of congenital adrenal hyperplasia, adrenal insufficiency, chronic pelvic pain and prostatitis, oncology (breast cancer) and in athletes. LC-MS/MS facilitates the specificity and limits of quantification required for the reliable analysis of steroids in human fluids, increasing diagnostic capabilities, particularly when steroid profiles are available

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Anal Biochem 2009 **388** (2) 179

Stabilization of human urine doping control samples

No abstract available. Review headings: Degradation effects on doping control urine samples; Hydrolysis of steroid conjugates; Increase of T in the free fraction; Formation of metabolic by-products; Production of boldenone; Formation of 19-norsteroids; Gonadotropins degradation; Human chorionic gonadotropin; Luteinizing hormone; Enzymatic shift and disappearance of EPO bands; Sources of microbial and enzymatic activities in doping control samples; Species of the normal microbial flora; Microbes of the urinary tract; Species of the indoor environment; Proteolytic enzymes; Stability studies of steroids and proteins; Steroids; Human chorionic gonadotropin; Luteinizing hormone; Erythropoietin; Inhibition of microbial growth and enzymatic action; Physical methods; Membrane filtration; Ultraviolet irradiation; Ultrasonication; Chemical methods; Boric acid; Sodium azide; Penicillin–streptomycin–amphotericin; Cycloheximide; Chloramphenicol; 2,3-Deydro-2-deoxy-*N*-acetyl-neuraminic acid; 4-Methoxyphenylsulfamate; Pepstatin; Phenylmethylsulfonyl fluoride; Pefabloc (AEBSF); Protease inhibitor cocktails and EDTA

2 Sports Doping - General

Badoud F, Grata E, Perrenoud L, Avois L, Saugy M, Rudaz S, Veuthey JL*// *Univ Lausanne Univ Geneva, Sch Pharmaceut Sci, 20 Blvd Yvoy, CH-1211 Geneva 4, Switzerland

J Chromatogr A 2009 **1216** (20) 4423

Fast analysis of doping agents in urine by ultra-high-pressure liquid chromatography-quadrupole time-of-flight mass spectrometry I. Screening analysis

Screening for a wide range of compounds is the starting point for the analysis of anti-doping analyses of urine samples. This initial procedure needs to be fast, generic and able to detect any sample that may contain a prohibited substance while avoiding false negatives and reducing false positive results. Investigations based on ultra-high-pressure liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry are described. Due to the high sensitivity of the technique, urine samples could be diluted 2-fold prior to injection. One hundred and three banned substances from various classes (such as stimulants, diuretics, narcotics, anti-estrogens) were determined on a C_{18} reversed-phase column in two gradients of 9 min (including two 3 min equilibration periods) for positive and negative electrospray ionisation and detected in the MS full scan mode. Automatic identification of compounds was based upon retention time and mass accuracy and with an automated tool for peak picking. The procedure was validated in respect of the International Standard for Laboratories described in the World Anti-Doping Code and was selective enough to conform to World Anti-Doping Agency recommendations. Additionally, the matrix effect on MS response was investigated on all analytes spiked in urine samples. Limits of detection ranged from 1 to 500 ng/ml, facilitating the identification of all investigated compounds in urine. If a sample tested positive during the screening, a fast additional preliminary step was carried out to reduce the number of confirmatory analyses

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Russ J Phys Chem A 2009 **83** (4) 513

Matrix effect on the determination of synthetic corticosteroids and diuretics by liquid chromatography-tandem mass spectrometry

The selective and reliable screening of corticosteroids and diuretics in human urine has been achieved employing high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Preparation of samples included extraction and evaporation of the organic extract under nitrogen. Content of the sample was analysed by HPLC combined with tandem mass spectrometry using electro-spraying ionization at atmospheric pressure with negative ion recording. Mass spectra of all compounds were recorded, and the characteristic ions, retention times, and detection limits were determined. The method was authenticated by investigating the degree of matrix suppression of ionization, extraction of analytes from human biological liquid, and the selectivity and specificity of determination

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Accredit Qual Assur 2009 **14** (6) 341

On the unacceptable reporting of results in doping control (Letter)

No abstract available

3 Steroids

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Russ J Phys Chem A 2009 **83** (4) 530

Determination of sulfates and glucuronides of endogenic steroids in biofluids by high-performance liquid chromatography/orbitrap mass spectrometry

High-performance liquid chromatography/high-resolution mass spectrometry using solid phase microextraction on a MEPS cartridge was employed to investigate the possibility of selective determination of testosterone and epitestosterone glucuronides in urine. By utilising the spectra of conjugates recorded for urine samples after hydrolysis as reference spectra, the influence of the biological matrix on the spectra of conjugated steroids may be accommodated. The conditions of fragmentation in the ion source were optimized for each analyte. This procedure was employed for analyzing real samples with different testosterone/epitestosterone ratios. Variations in conjugate contents and qualitative changes in the steroid profile of endogenic compounds were recorded

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Trends Anal Chem 2009 **28** (6) 718

Fluorescence site-encoded DNA addressable hapten microarray for anabolic androgenic steroids

Immunochemical screening of small organic molecules based on the use of a hapten microarray is reported. By employing DNA-directed immobilization strategies, it has been possible to convert a DNA chip into a hapten microarray by benefitting from the structural and electrostatic homogeneous properties of DNA. The hapten microarray employs hapten-oligonucleotide probes rather than proteins thus avoiding the shortcomings of preparing stochiometrically-defined protein-oligonucleotide bioconjugates. As proof of concept, a microarray for analysis of anabolic androgenic steroids has been developed. The microchip is capable of determining several illegal substances with sufficient precision to be employed as a screening method in accordance with the regulations of the World Anti-Doping Agency for sport and the European Commision for food safety. The data confirm the extensive possibilities of the DNA chip, and, in this instance, illustrate a procedure to develop hapten microarrays for the immunochemical analysis of small organic molecules including anabolic androgenic steroids

4 Peptides

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Russ J Phys Chem A 2009 **83** (4) 520
HPLC-MS/MS investigation of biochemical markers for the disclosure of erythropoietin abuse in sports
Erythropoietin (EPO) is a polypeptide hormone which is prohibited in sports. Abuse of EPO was investigated using high-performance liquid chromatography combined with tandem mass spectrometry (HPLC-MS/MS). The potential influence of EPO on the asymmetric dimethylarginine (ADMA)-dimethylargininedime-thylaminohydrolase (DDAH)-NO-synthase system was confirmed. Alterations to this enzyme system might serve as indirect biochemical markers of the presence of the prohibited EPO drug. Concentrations of biochemical markers varied from 10 to 40 µg/ml for ADMA and symmetrical DMA (SDMA) and from 0.5 to 10 µg/ml for arginine and citrulline. However, following a single intravenous administration of r-HuEPO (Epocrin, 2000 ME/day) to two volunteers there were consistent increases ADMA, SDMA, arginine, and citrulline concentrations to 40-270 µg/ml, 40-240 µg/ml, 10-60 µg/ml, and 12-140 µg/ml, respectively. Simultaneous increases in arginine, methylarginines, and citrulline contents might be an indirect marker of EPO abuse. High-performance liquid chromatography combined with tandem mass spectrometry is recommended for fast screening analysis

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Haematologica 2009 **94** (6) 888
Detection of continuous erythropoietin receptor activator in blood and urine in anti-doping control (Letter)
No abstract available

6 CNS Agents

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J Anal Toxicol 2009 **33** (3) 162
Elimination of ephedrines in urine following administration of a Sho-seiryu-to preparation
One of the most common Traditional Chinese Medicine preparations for the treatment of colds is Sho-seiryu-to which contains Ephedrae Herba. However, the major ingredients of Ephedrae Herba, ephedrines, are banned substances on the World Anti-Doping Agency (WADA) list. The aim of this research was to determine the elimination of urinary ephedrines following administration of Sho-seiryu-to preparation and to investigate the possibility of positive ephedrines test results in urine. Six healthy volunteers took a single 2.5-g dose of concentrated Sho-seiryu-to preparation and all urine was collected for 48 h. The concentrations of urinary ephedrines were analyzed by high-performance liquid chromatography and the elimination half-life of the ephedrines was determined. Folllowing a single dose of Sho-seiryuto preparation, ephedrine and cathine (norpseudoephedrine), the prohibited substances of the WADA, were excreted in the urine. The peak concentration of ephedrine was 3.88 +/- 1.87 mg/ml (mean +/- SD) and this was lower than the WADA permitted value (10 mg/ml). Estimated elimination half-lives of ephedrine, norephedrine, pseudoephedrine, and norpseudoephedrine following administration of this preparation were 5.3 +/- 1.2, 4.9 +/- 0.9, 4.4 +/- 1.0, and 5.4 +/- 1.8 h, respectively. Therefore, urine concentrations would not violate the antidoping rules following a single dose of Sho-seiryu-to preparation. However, an applied multiple-dose study after administering the preparation for three times per day for three days resulted in a positive urine ephedrine result (13.7 mg/ml). Athletes should be mindful when taking more than a single dose of Sho-seiryu-to preparation

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J Chromatogr A 2009 **1216** (28) 5340
Analysis of -agonists and -blockers in urine using hollow fibre-protected liquid-phase microextraction with *in situ* derivatization followed by gas chromatography/mass spectrometry
The analysis of β-agonists and β-blockers in urine was accomplished employing hollow fibre-protected liquid-phase microextraction (HF-LPME) with *in situ* derivatization followed by gas chromatography/mass spectrometry (GC/MS). Extraction and derivatization of compounds of interest with methylbenzol and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) in HF-LPME mitigates the drawbacks of considerable time-consuming and tedious operation whilst improving enrichment. Optimized conditions comprised extraction for 20 min at 35°C with 5.0 µl of mixed extraction solvent (methylbenzol/MSTFA = 1:1, v/v) with stirring speed of 925 rpm in 5.0 ml sample under pH 12.0 and 14% (w/v) NaCl. The technique provided very wide linear ranges (0.25-400 ng/ml) and low detection limits in the range of 0.08-0.10 ng/ml for clenbuterol, metoprolol and propranolol while enrichment factors reached up to 256. The compounds were analysed in spiked urine with high extraction efficacy (93.79-109.04% recoveries) and precision (<9.70% RSD). Satisfactory results were obtained for metoprolol in real human urine samples following a single-dose administration of 50 mg after 36 h. The proposed procedure only requires a few microliters of organic solvent and derivatizing agent, the operation is simple, convenient and rapid for the trace analysis of β-agonists and β-blockers in biological fluids. The procedure may be readily applied for high sample throughput. It is proposed that the data will facilitate the monitoring of β-agonists and β-blockers in the competition sports

7 Equine

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Chromatographia 2009 **69** (9-10) 923
Detection and confirmation of ginsenosides in horse urine by GC-MS and LC-MS
For thousands of years, ginseng has been used by the Chinese as a traditional herbal medicine. There is an increasing concern about its abuse in both human and animal sports in respect of its increasing popularity in the use of ginseng preparations worldwide both as natural remedies and food supplements. The pharmacological properties of ginseng are considered to result from its major constituents, ginsenosides. The aim of this study was develop a procedure for the detection and confirmation of a number of ginsenosides in equine urine. Intact ginsenosides were detected and confirmed at 5-100 ng/ml by LC-MS^2, and two deglycosylation metabolites, namely protopanaxadiol and protopanaxatriol, were detected and confirmed at 2 ng/ml by GC-MS^2 after trimethylsilylation. Both techniques were applied to the investigation of the *in vitro* metabolism of ginsenosides Rg1 and Rb1 and the *in vivo* urinary metabolites after oral administration of Rg1 to horses. Results produced demonstrate the very first evidence for the existence of the metabolites, Rg1 and protopanaxatriol, as glucuronides in urine

8 Recreational Drugs - General

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J Clin Psychopharmacol 2009 **29** (3) 248
Evaluation of a urine on-site drugs of abuse screening test in patients admitted to a psychiatric emergency unit
A urinary commercially available on-site drugs of abuse screening test device commonly employed for admittances to a psychiatric emergency unit was investigated in respect of usefulness and reliability. Amphetamines, benzodiazepines, cannabis, cocaine, and opiates were analysed in urine samples from 262 emergency psychiatric admittances representing 217 patients. Initially, samples were screened by nurses at the psychiatric department, followed by 2 technicians in the laboratory, and finally, analyzed by liquid chromatography/mass spectrometry. Results of 45.8% of the screening tests were correctly negative for all 5 drugs/drug groups tested, whereas those of 29.4% were correctly positive for 1 or several drugs/drug groups and correctly negative for the others. Consequently, in sum, 75.2% were accurate for all 5 drugs/drug groups. Overall, the sensitivities (42.9%-90.0% for the various drug groups) were lower than the specificities (92.7%-100.0%). Accuracies were 86.3% for benzodiazepines, 92.4% for cannabis, 94.7% for opiates, and 97.0% for amphetamines. Cocaine was not found in any of the samples. For cannabis, the accuracy was greater with the laboratory technicians than nurses. On-site screening testing must not be considered as the final presumption on the intake of recreational drugs and should be interpreted with caution

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J Chromatogr B 2009 **877** (11-12) 1162

A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine

The simultaneous detection of 10 plant-derived psychoactive substances (atropine, *N*,*N*-dimethyltryptamine, ephedrine, harmaline, harmine, ibogaine, lysergic acid amide, psilocin, scopolamine and yohimbine) in urine has been achieved by the development of a sensitive and specific LC-MS/MS technique. A readily accessible procedure suitable for application in clinical intoxication cases was facilitated by direct injection of urine diluted with 3 deuterated internal standards. Separation was accomplished using reversed phase chromatography and gradient elution with a total analysis time of 14 min. Electrospray ionization was employed and ions monitored in the positive selected reaction monitoring mode. The calibration curves were linear (r^2>0.999) and the total imprecision at high (1000 µg/l) and low (50 µg/l) substance concentrations were 4.9-13.8% and 8.3-26%, respectively. Infusing the analytes post column and injecting matrix samples demonstrated limited influence by ion suppression. The multi-component procedure proved to be suitable for investigation of authentic cases of intoxication with plant-derived psychoactive drugs and was demonstrated to include the clinically relevant concentration ranges

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J Anal Toxicol 2009 **33** (5) 243

Simultaneous quantification of methadone, cocaine, opiates, and metabolites in human placenta by liquid chromatography-mass spectrometry

The quantitative analysis of human placenta for methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, cocaine, benzoylecgonine, 6-acetylmorphine, morphine, and codeine by a validated technique involving liquid chromatography-ion trap mass spectrometry is described. Samples of placentas (1g) were homogenized and subjected to solid-phase extraction. A Synergi Polar RP column was employed for chromatographic separation with a gradient of 0.1% formic acid and acetonitrile. The method was linear from 10 to 2000 ng/g for methadone and 2.5 to 500 ng/g for other analytes. Limits of detection were 0.25-2.5 ng/g, imprecisions < 9.1%CV, analytical recoveries 84.4-113.3%, extraction efficiencies > 46%, matrix effects -8.0-129.9%, and process efficiencies 24.2-201.0%. Five specimens of placentas from opioid-dependent women receiving methadone pharmacotherapy with methadone doses ranging from 65 to 95 mg on the day of delivery were analysed. These represent the first data on placental concentrations of methadone and metabolites after controlled drug administration. Detection of other common drugs of abuse in placenta will enhance our knowledge of the applicability of this matrix for detection of *in utero* drug exposure and disposition of drugs in the maternal-fetal dyad

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Clin Chem 2009 **55** (6) 1203

Chemoinformatic methods for predicting interference in drug of abuse/toxicology immunoassays

Cross-reacting compounds able to bind to the antibodies in a manner similar to the target molecule(s) limit screening immunoassays routinely employed for drug of abuse (DOA)/toxicology analysis. There has been little systematic analysis with computational tools to predict cross-reactive compounds. Routinely employed molecular similarity techniques enabled calculation of structural similarity for a wide range of compounds (prescription and over-the-counter medications, illicit drugs, and clinically significant metabolites) to the target molecules of DOA/toxicology screening assays. Various molecular descriptors (MDL public keys, functional class fingerprints, and pharmacophore fingerprints) and the Tanimoto similarity coefficient were employed. The data were then compared with cross-reactivity data in the package inserts of immunoassays marketed for *in vitro* diagnostic use. Previously untested compounds predicted to have a high probability of cross-reactivity were investigated. Molecular similarity calculated using MDL public keys and the Tanimoto similarity coefficient demonstrated a strong and statistically significant separation between cross-reactive and non-cross-reactive compounds. The result was experimentally authenticated by discovery of additional cross-reactive compounds based on computational predictions. Computational techniques are amenable in respect of rapid screening of databases of drugs, metabolites, and endogenous molecules and may be useful for identifying cross-reactive molecules that might otherwise unsuspected. These techniques may also prove beneficial in concentrating cross-reactivity testing on compounds with high similarity to the target molecule(s) and limiting testing of compounds with low similarity and very low probability of cross-reacting with the assay

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Appl Spectrosc 2009 **63** (7) 742

Rapid classification of simulated street drug mixtures using Raman spectroscopy and principal component analysis

It is important for criminal investigations and prosecution to have the ability to accurately and noninvasively analyze illicit drugs. Current procedures involve significant sample pretreatment and most are destructive. A procedure based on Raman spectroscopy to classify simulated street drug mixtures containing one drug component and up to three cutting agents including those routinely found in confiscated illicit street drug mixtures has been developed. Spectra were provided by both a homebuilt instrument employing a HeNe laser and on a handheld commercial instrument with a 785 nm light source. Mixtures were assembled with drug concentrations ranging from 10 to 100%. Optimal preprocessing for the data set included truncating, Savitzky-Golay smoothing, normalization, differentiating, and mean centering. By employing principal component analysis (PCA), it was possible to resolve the spectral differences between benzocaine, lidocaine, isoxsuprine, and norephedrine and accurately classify them.

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Anal Chem 2009 **81** (11) 4382

Determination of drugs of abuse in airborne particles by pressurized liquid extraction and liquid chromatography-electrospray-tandem mass spectrometry

The first analytical technique specifically developed for the multianalyte determination of several drugs of abuse and their metabolites in air is described. The procedure comprises pressurized liquid extraction (PLE) of atmospheric particles collected by means of high volume sampler equipped with quartz microfiber filters and subsequent analysis of the extracts by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Five different chemical classes (cocainics, amphetamine-like compounds, opioids, cannabinoids, and lysergic compounds) including 17 different compounds were determined by means of this methodology. Acquisition was performed in the selected reaction monitoring (SRM) mode recording two transitions per compound (except for amphetamine). Quantitation employed the internal standard technique using surrogated deuterated standards. The method has been validated in terms of linearity, accuracy, repeatability and sensitivity with satisfactory results. Absolute recoveries were greater than 50% for most investigated drugs. Method precision demonstrated relative standard deviations (RSD) less than 13% for all compounds with the exception of cannabinoids. Limits of determination ranged from 0.35 pg/m^3 (for 2-oxo-3-hydroxy-LSD) to 22.55 pg/m^3 (for 11-nor-9 carboxy THC). The optimized procedure was applied to and in part, validated by the analysis of ambient air samples (fine grain-size particulates, $PM_{2.5}$) collected at two urban background sites in Barcelona and Madrid (Spain). Results demonstrated the presence of cocaine, benzoilecgonine, tetrahydrocannabinol, ecstasy, amphetamine, methamphetamine, and heroin in some or all of the samples investigated. The highest mean daily levels were represented by cocaine (850 pg/m^3) followed by heroin (143 pg/m^3)

9 Stimulants

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Forensic Sci Int 2009 **186** (1-3) 63

Pharmacokinetics of 'party pill' drug *N*-benzylpiperazine (BZP) in healthy human participants

Since the late 1990's, 'party pills' containing benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) have been widely available. To date, there is no liertaure describing the pharmacokinetics of these drugs in humans. Following a 200mg oral dose of BZP, human plasma concentrations of BZP were measured in blood and urine samples taken from healthy adults (n = 7) over 24h. Plasma concentrations of BZP were found to peak at 262 ng/ml (C_{max}) and 75min (T_{max}). Plasma concentrations of the major metabolites of BZP, 4-OH BZP and 3-OH BZP, were found to peak at 7 ng/ml (at 60 min) and 13 ng/ml (at 75 min) respectively. The elimination half-life ($t_{1/2}$) for BZP was found to be 5.5h and clearance (Cl/F) was found to be 99 l/h. These

results demonstrate that BZP may be detectable in plasma for up to 30 h following an oral dose. Furthermore, several urinary metabolites can be detected

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J Chromatogr A 2009 **1216** (18) 4083
Orthogonal array optimization of microwave-assisted derivatization for determination of trace amphetamine and methamphetamine using negative chemical ionization gas chromatography-mass spectrometry
Orthogonal array design (OAD) was employed to optimize microwave-assisted derivatization (MAD) in order to analyse trace amphetamine (AM) and methamphetamine (MA) by negative chemical ionization gas chromatography-mass spectrometry (NCI GC-MS). The derivatization reagent was 2,3,4,5,6-pentafluorobenzoyl chloride (PFBC). Experimental parameters including solvent, microwave power, and irradiation time at four-levels were studied in 16 trials by OAD_{16} (4^4). The significance of these factors was examined using analysis of variance (ANOVA) and percent contribution (PC). Solvent was demonstrated statistically as a chief factor; microwave power and irradiation time were secondary factors. Under the optimum condition, calibration curve of AM was linear over a range from 0.01 to 100 ng/ml with correlation coefficient 0.9988, and MA from 0.1 to 1000 ng/ml with correlation coefficient 0.9951. The limit of detection (LOD) is 1.20 pg/ml for AM and 13.04 pg/ml for MA. Analysis of urine samples from amphetamine-type stimulants (ATS)-abusing suspects was employed to test the applicability of the method. Therefore, the OAD method not only optimizes the MAD condition for determination of trace AM and MA but also identifies the effects of solvent, microwave power and irradiation time on the MAD performance

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Forensic Sci Int 2009 **187** (1-3) 19
A study of the thermal decomposition of adulterated cocaine samples under optimized aerobic pyrolytic conditions
By employing conditions that are relevant to the smoking of illicit cocaine by humans, the pyrolysis behaviour of pure cocaine base as well as the influence of various additives was investigated. An aerobic pyrolysis device was developed. Firstly, optimisation of some basic experimental parameters of the pyrolysis was performed, i.e., the furnace temperature, the sampling start time, the heating period, the sampling time, and the air-flow rate through the system. Secondly, the investigation centred on the volatile products formed during the pyrolysis of a pure cocaine free base and mixtures of cocaine base and adulterants. Adulterants included the anaesthetics lidocaine, benzocaine, procaine, the analgesics phenacetine and paracetamol, and the stimulant caffeine. Complete volatilization of the samples was achieved under the applied experimental conditions, i.e., the residuals of the studied compounds were not detected in the pyrolysis cell. Volatilization of the pure cocaine base demonstrated that the cocaine recovery available for inhalation (adsorbed on traps) was approximately 76%. Analyses of the smoke condensate by GC-MS and NMR demonstrated the presence of some additional cocaine pyrolytic products, such as anhydroecgonine methyl ester (AEME), benzoic acid (BA) and carbomethoxycycloheptatrienes (CMCHTs). The thermal behaviour of the cocaine was influenced by the addition of different cocaine-adulterant mixtures, the most significant of which was paracetamol. Total recovery of the cocaine (adsorbed on traps and in a glass tube) from the 1:1 cocaine-paracetamol mixture was found to be only 3.0+/-0.8%, compared with 81.4+/-2.9% for the pure cocaine base. Other adulterants exhibited less-extensive effects on the recovery of cocaine. However, the pyrolysis of the cocaine-procaine mixture led to the formation of some unique pyrolytic products of which two were identified as *para*-aminobenzoic acid (*p*-ABA) and 2-(diethylamino)ethylbenzoate (DEAEB)

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J Chromatogr A 2009 **1216** (24) 4824
Preparation of ionic liquid based solid-phase microextraction fiber and its application to forensic determination of methamphetamine and amphetamine in human urine
A new solid-phase microextraction (SPME) technique employing an ionic liquid (IL) has been developed. A reusable IL-based SPME fiber was constructed for the first time by fixing IL by means of cross-linkage of IL impregnated silicone elastomer on the surface of a fused silica fiber. 1-Ethoxyethyl-3-methylimidazloium bis(trifluoromethane) sulfonylimide ([EeMim][NTf_2]) ionic liquid was used as test compound. The prepared fiber was utilised for the forensic headspace determination of methamphetamine (MAP) and amphetamine (AP) in human urine samples. Critical extraction parameters were investigated and optimized including the concentration of salt and base in sample matrix, extraction temperature and extraction time. When combined with gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring (SIM) mode, the new technique showed good linearity in the range of 20-1500 μg/l, good repeatability (RSD<7.5% for MAP, and <11.5% for AP, $n = 6$), and low detection limits (0.1 μg/l for MAP and 0.5 μg/l for AP). Human urine samples were employed to evaluate the feasibility of the method. Whereas IL-based SPME is still at an early stage of development, the results produced by this work demonstrate that it is a promising simple, fast and sensitive sample preparation method

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J Am Soc Mass Spectrom 2009 **20** (5) 891
Screening of cocaine and its metabolites in human urine samples by direct analysis in real-time source coupled to time-of-flight mass spectrometry after online preconcentration utilizing microextraction by packed sorbent
Fast screening of recreational drugs has been achieved with microextraction by packed sorbent (MEPS) coupled to mass spectrometric detection. Sorbents for the MEPS were C8 (octyl-silica, useful for nonpolar to moderately polar compounds), ENV^+ (hydroxylated polystyrene-divinylbenzene copolymer, for extraction of aliphatic and aromatic polar compounds), Oasis MCX [sulfonic-poly(divinylbenzene-*co*-*N*-polyvinyl-pyrrolidone) copolymer], and Clean Screen DAU (mixed mode, ion exchanger for acidic and basic compounds). The purpose of the study was the fast extraction and preconcentration of the drugs and rapid analysis employing a time-of-flight (TOF) mass spectrometer as the detector with direct analysis in a real-time (DART) source. The association of an analysis time of less than 1 min with accurate mass of the first monoisotopic peak of the analyte and the relative abundances of the peaks in the isotopic clusters provided reliable data for identification. In addition, the research aimed to show that it is possible to quantify the analyte of interest using a DART source when an internal standard is employed. Of the 4 sorbents investigated, Clean Screen DAU performed best for adsorption of analytes from urine. When Clean Screen DAU was employed to extract spiked samples containing the drugs, linearity wasshown for ecgonine methyl ester, benzoylecgonine, cocaine, and cocaethylene with average ranges of: 65-910, 75-1100, 95-1200, and 75-1100 ng/ml ($n = 5$), respectively. The limits of detection (LOD) for ecgonine methyl ester, benzoylecgonine, cocaine, and cocaethylene were 22.9 ng/ml, 23.7 ng/ml, 4.0 ng/ml, and 9.8 ng/ml respectively, utilising a signal-to-noise ratio of 3:1

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J Anal Toxicol 2009 **33** (3) 170
Low concentrations of methamphetamine detectable in urine in the presence of high concentrations of amphetamine
Subsequent analysis for the presence of methamphetamine was carried out on 22 urine specimens reported by military drug-testing laboratories for the presence of high concentrations of amphetamine. The 22 urine specimens contained concentrations of amphetamine in the range of 28,028 to 241,142 ng/ml. The specimens were also investigated for the respective isomeric ratio of *d*-(*S*) and *l*-(*R*) amphetamine and methamphetamine. The results indicate that urine specimens containing high concentrations of amphetamine where the ratio of methamphetamine to amphetamine is less than 0.5% with similar isomeric distribution of *d*-(*S*) and *l*-(*R*) amphetamine and methamphetamine may not necessarily provide evidence of polydrug use

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J Chromatogr A 2009 **1216** (18) 4063
Rapid identification and quantification of methamphetamine and amphetamine in hair by gas chromatography/mass spectrometry coupled with micropulverized extraction, aqueous acetylation and microextraction by packed sorbent
A rapid identification and quantification technique for the toxicological analysis of methamphetamine and amphetamine in human hair has been developed employing gas chromatography/mass spectrometry coupled with a novel combination of micropulverized extraction, aqueous acetylation and microextraction by packed sorbent (MEPS) named MiAMi-GC/MS. A washed hair sample (1-5 mg) was micropulverized for 5 min in a 2 ml plastic tube with 250 μl of water. Anionic interferences were adsorbed using an anion-exchange sorbent. Following removal of the residue with a membrane-filter unit, sodium carbonate and acetic anhydride were admixed in turn. At room temperature, acetylation was completed in approximately 20 min. Acetylated

compounds in the reaction liquid were concentrated to an octadecylsilica sorbent packed in the needle of a syringe by a CombiPAL autosampler. Elution was achiev with 50 µl of methanol and the entire eluate injected into a gas chromatograph using a programmable temperature vaporizing (PTV) technique. The time required for sample preparation and GC/MS analysis was approximately 1 h from a washed hair sample, and an evaporation process was not necessary. Ranges for quantification were 0.20-50 (ng/mg) each for methamphetamine and amphetamine employing 1 mg of hair. Accuracy and relative standard deviation (RSD) were evaluated intraday and interday at three concentrations, and the results were within the limit of a guidance issued by U.S. Food and Drug Administration. For identification, full-scan mass spectra of methamphetamine and amphetamine were produced using 5 mg of fortified hair samples at 0.2 ng/mg. The extraction device of MEPS was durable for at least 300 extractions, whereas the liner of the gas chromatograph required replacing after 20-30 times use. Carry over was estimated to be about 1-2%. This sample-preparation method coupled with GC/MS is rapid and less labor-intensive when compared with conventional methods

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Sci Justice 2009 **49** (2) 94

Comparative analysis of 1-phenyl-2-propanone (P2P), an amphetamine-type stimulant precursor, using stable isotope ratio mass spectrometry presented in part as a poster at the 2nd meeting of the Joint European Stable Isotope User Meeting (JESIUM), Giens, France, September 2008

The isotope ratios of amphetamine type stimulants (ATS) result from both the precursor and the synthetic pathway. Illicit production of amphetamine and methamphetamine often employs 1-phenyl-2-propanone (P2P, benzylmethylketone) as a precursor. The variation of the isotope ratios within precursor samples of one manufacturer was compared seized samples of unknown sources. $\delta^{13}C_{V\text{-}PDB}$, $\delta^{2}H_{V\text{-}SMOW}$ and $\delta^{18}O_{V\text{-}SMOW}$ isotope ratios were determined using elemental analysis (EA) and gas chromatography (GC) coupled to an isotope ratio mass spectrometer (IRMS). Considerable differences were noted between all seized samples and the the data of the samples of one legal manufacturer. The data demonstrate that IRMS provides promising reliability in differentiating between precursors from different manufacturers for the clandestine production of ATS and identifying corresponding sources

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Forensic Sci Int 2009 **187** (1-3) 87

Structure elucidation of a new designer benzylpiperazine: 4-Bromo-2,5-dimethoxybenzylpiperazine

A first seizure has been made of a new designer benzylpiperazine in Germany. Following gas chromatography-mass spectroscopy (GC-MS), product ion spectroscopy (GC-MS/MS), and nuclear magnetic resonance (NMR) spectroscopy the compound was identified as 4-bromo-2,5-dimethoxybenzylpiperazine. The structure of the new benzylpiperazine was ultimately proven by two-dimensional NMR correlations and by GC-MS after synthesis of two of the possible isomers from commercially available starting materials. Mass spectroscopic data following liquid chromatography-mass spectroscopy (LC-MS/MS) using electrospray ionization (ESI) as well as ultraviolet (UV) spectral data of the new compound are presented. A small quantity of the new benzylpiperazine was seized in very high purity along with other also very pure designer drugs in Hamburg, Germany

10 Hallucinogens

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Forensic Sci Int 2009 **188** (1-3) 68

Analysis of fatty acids in Ecstasy tablets

Lubricants often employed in the production of Ecstasy tabletsare stearates based on fatty acids. The fatty acids are added at the initial stage of tablet production. Therefore, the profile does not change once the compression is performed. Consequently, analysis of fatty acids provides useful information for drug intelligence authorities. To provide the necessary data, an appropriate analytical technique was developed to improve results already obtained by routine methods. Due to the small quantity of the fatty acids in Ecstasy tablets (approximately 3%) the research targeted their extraction and concentration. Two different methods were employed: (1) liquid/liquid extraction using dichloromethane followed by derivatisation and (2) *in situ* transesterification using bortrifluoride. GC-MS was utilised for the analyses. The two methods were optimized and applied to eight Ecstasy seizures, in order to choose one of the procedures for its application to a large Ecstasy sample set. Procedures were compared in respect of the number of peaks detected and sample amount needed, reproducibility and other technical aspects

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Anal Chim Acta 2009 **641** (1-2) 89

Evaluation of principal cannabinoids in airborne particulates

Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CND) and cannabinol (CNB) are the primary active components in cannabis preparations. Analysis of airborne particulates was achieved by employing a technique comprising soot extraction by ultrasonic bath, purification by solvent partitioning, derivatization with *N*-(*t*-butyldimethylsilyl)-*N*-methyl-trifluoroacetamide, and separation/detection through gas chromatography coupled with tandem mass spectrometry. When optimized, the method was found suitable for measuring the three psychotropic substances at concentrations ranging from ca. 0.001 to ca. 5.0 ng/cm^{-3} of air, with recoveries always higher than 82%, accuracy >7.3% and precision >90%. The method was applied to ambient air in Rome and Bari, Italy, where all three compounds were identified. However, in Algiers, Algeria, only cannabinol, the most stable compound in the atmosphere, exceeded the limit of quantification of the method. In general, the relative percentages of the three cannabinoids reproduced those typical of the *Cannabis sativa* plant. These were very different from those found in human blood, urine and sweat

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J Anal Toxicol 2009 **33** (3) 143

Multiple drug ingestion by Ecstasy abusers in the United States

Young adults attending "Rave" parties recreational drugs of choice are typically of the Ecstasy-type such as 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA). There are few toxicological details regarding Ecstasy usage by individuals undergoing monitoring in other settings in the United States. This investigation was to determine the prevalence and patterns of licit and illicit drugs in urine specimens of Ecstasy users. A survey of laboratory data over the years 2005-2007 revealed that 198 urine specimens were confirmed positive (cutoff concentration 100 ng/ml) for MDMA and/or MDA from the following types of donors (#positive specimens): Correctional (159); sports (19); workplace (9); pain patients (8); and special test requests (3). Of these, 122 (61.6%) were positive for MDMA and MDA, 70 (35.4%) were positive for MDMA, and 6 (3.0%) were positive for MDA. Most of the specimens (84.3%) contained multiple drugs and/or metabolites in addition to MDMA and MDA. The median number of drugs/metabolites reported for these Ecstasy users was 5 (range, 1-9). In addition to MDMA/MDA, the most commonly identified drug groups were cannabis (THCCOOH) (61.6%); amphetamine/ methamphetamine (38.4%); benzoylecgonine (30.8%); diazepam-related (9.6%); opiates (7.1%); alprazolam (5.6%); and others (5.6%). Whereas multidrug ingestion appears to be prevalent amongst Ecstasy users, caution is recommended in interpretation because the drug in the United States and Canada frequently contains methamphetamine and other active substances. Therefore, multidrug use may not have been intentional

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Anal Sci 2009 **25** (4) 517

Ecstasy analysis by monolithic materials-capillary electrochromatography

The analysis of 3,4-methylenedioxymethamphetamine (MDMA) in Ecstasy tablets and its metabolites in urine samples were investigated by means of monolithic columns prepared and employed with capillary electrochromatography. Cationic and neutral monolith columns proved to have better efficiencies than anionic monoliths. Neutral butyl methacrylate (BMA) monolith columns resulted in symmetrical peaks with the highest efficiency, high resolution and short analysis times. The procedure employing BMA monolithic columns resulted in detection limits for MDMA and its metabolites at 1 µg/ml with excellent intra-day and inter-day precision and linearity from 7.5 to 100 µg/ml (r^2 > or = 0.99). The main component in Ecstasy tablets was found to be MDMA while 4-hydroxy-3-methoxymethamphetamine was the major metabolite in urine samples. High recoveries (97% for MDMA from tablets and 84-102% for its metabolites from urine samples) resulted from simple ultrasonic extraction for tablets and liquid-liquid extraction for urine samples

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J Anal Toxicol 2009 **33** (5) 253
Validated ultra-performance liquid chromatography-tandem mass spectrometry method for analyzing LSD, *iso*-LSD, *nor*-LSD, and O-H-LSD in blood and urine
The Royal Canadian Mounted Police Forensic Science and Identification Services required a confirmatory technique for lysergic acid diethylamide (LSD). An ultra-performance liquid chromatography-tandem mass spectrometry method was authenticated for the identification and quantitation of LSD, *iso*-LSD, *N*-demethyl-LSD (*nor*-LSD), and 2-oxo-3-hydroxy-LSD (O-H-LSD). Relative retention time and ion ratios were employed as identification parameters. Limits of detection (LOD) in blood were 5 pg/ml for LSD and *iso*-LSD and 10 pg/ml for *nor*-LSD and O-H-LSD. The LOD was 10 pg/ml for all analytes in urine. In blood and urine, limits of quantitation (LOQ) were 20 pg/ml for LSD and *iso*-LSD and 50 pg/ml for *nor*-LSD and O-H-LSD. The procedure was linear, accurate, and precise from 10 to 2000 pg/ml in blood and 20 to 2000 pg/ml in urine for LSD and *iso*-LSD and from 20 to 2000 pg/ml in blood and 50 to 2000 pg/ml in urine for nor-LSD and O-H-LSD with a coefficient of determination (r^2 > or = 0.99). The technique was employed with blinded biological control samples and biological samples taken from a suspected LSD user. The first detection of O-H-LSD in blood from a suspected LSD user is reported

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J Anal Toxicol 2009 **33** (5) 283
A comparison between experimental and authentic blood/serum ratios of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine
The blood-to-serum distribution (B/S ratio) of 3,4-methylenedioxymethamphetamine (MDMA) and its major metabolite 3,4-methylenedioxyamphetamine (MDA) have been compared. For 16 healthy volunteers participating in a controlled driving experiment (authentic specimens), B/S ratios were analysed by liquid chromatography-tandem mass spectrometry analysis after liquid-liquid extraction as a function of the hematocrit value (experimental specimens) and in blood and corresponding serum samples (n = 63). A regression analysis to calculate the B/S ratio was performed followed by an analysis of covariances (ANCOVA). A linear relationship between the hematocrit value and the B/S ratio of both MDMA and MDA was produced from the experimental data. With MDMA, the regressions produced mean B/S ratios of 1.22 and 1.26 for the experimental setting and the authentic samples, respectively. For MDA, the analysis produced slopes of 1.15 and 1.27 for the experimental setting and field study, respectively. ANCOVA demonstrated that the method of determination (experimental *vs*. authentic specimens) did not influence the resulting slopes. A conversion factor of 0.80 may provide an satisfactory estimate to derive the serum concentration for MDMA when only the concentration in whole blood is known. However, a similar factor could not be derived for MDA because of its very low levels in authentic samples

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J Anal Toxicol 2009 **33** (5) 266
Determination of 4-hydroxy-3-methoxymethamphetamine as a metabolite of methamphetamine in rats and human liver microsomes using gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry
4-Hydroxy-3-methoxymethamphetamine (HMMA) is the main metabolite of 3,4-methylenedioxymethamphetamine (MDMA). An investigation was carried out to determine whether methamphetamine (MA) metabolism resulted in similar metabolite. MA was intravenously administered to rats and plasma, urine, and bile were collected periodically. Gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry detected HMMA together with MA and its main metabolites, amphetamine and 4-hydroxymethamphetamine in rat plasma, urine, and bile. Furthermore, HMMA was produced when MA was incubated with human liver microsomes. When humans have consumed MA, HMMA may be produced as a metabolite but the amount of HMMA would be small compared with that of MA, amphetamine, or 4-hydroxymethamphetamine. The present study should assist in the determination the type of drug consumed

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J Chromatogr A 2009 **1216** (18) 4090
Determination of -hydroxybutyric acid in serum and urine by headspace solid-phase dynamic extraction combined with gas chromatography-positive chemical ionization mass spectrometry
Gamma-hydroxybutyric acid is an emerging recreational drug. In addition to relaxation and euphoria, it results in hypnosis and unconsciousness. Consequently, it is misused as drug of abuse and at drug-facilitated sexual assaults. An automated and simple method for quantitation of γ-hydroxybutyric acid in serum and urine was optimized and validated. Five hundred microliters sample volume are used for both types of specimen. Acid catalyzed conversion of γ-hydroxybutyric acid to the corresponding γ-butyrolactone is employed. In addition, the technique was based on headspace solid-phase dynamic extraction coupled with gas chromatography-mass spectrometry. The extraction process was achieved by repeated aspiration and ejection of the headspace through a steel cannula which was coated on the inside with a polydimethylsiloxane sorbent. By employing this method, absorption of analyte molecules by the sorbent was achieved. The effects of sorbent type, incubation temperature, number of extraction strokes, injection port temperature and injection flow speed on extraction recovery were examined. The validation exhibited good accuracy with a bias less than +/-5%. Intra- and interday precision determined at 10, 50 and 150 µg/ml for each matrix were in following ranges: 1.96-3.49% (intraday, serum), 2.38-4.31% (intraday, urine), 2.33-5.13% (interday, serum) and 2.53-5.64% (interday, urine). The technique demonstrated good linearity between 2 and 200 µg/ml yielding coefficients of determination r^2 > or = 0.9985. The limits of detection were determined at 0.16 µg/ml for serum and 0.17 µg/ml for urine. This technique presents a fast, solvent-free and widely automated extraction process. It was successfully applied to toxicological routine analysis and therapeutic drug monitoring

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Forensic Sci Int 2009 **187** (1-3) 58
The profiling of MDMA tablets: A study of the combination of physical characteristics and organic impurities as sources of information
Different sets of characteristics may be employed when profiling MDMA tablets. Physical properties (i.e. post-tabletting characteristics) of MDMA tablets may be measured. Data provide preliminary profiling data that may be valuable in a first stage of an investigation. However organic impurities (i.e. pre-tabletting characteristics) are usually required as more reliable information, particularly for presentation of judicial evidence. This paper examines the added value of combining pre- and post-tabletting characteristics of seized MDMA tablets. Post-tabletting links were provided by organic impurities analyses in approximately half of the investigated cases. In the remaining cases, post-tabletting batches (post-TBs) were divided into several pre-tabletting batches (pre-TBs), thereby supporting the hypothesis that several production batches of MDMA powder (pre-TBs) were employed to produce one single post-TB (i.e. tablets having the same shape, diameter, thickness, weight and score; but different organic impurities composition). In respect of the findings, the hypotheses were discussed through illustrating examples. To conclude, both sets of characteristics were found relevant alone and combined together. They provide specific information regarding illicit MDMA production and trafficking

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Clin Chem 2009 **55** (6) 1188
Intra- and intersubject whole blood/plasma cannabinoid ratios determined by 2-dimensional, electron impact GCMS with cryofocusing
Concentrations of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) in whole blood are approximately half of those in plasma. This results from high plasma protein binding and poor cannabinoid distribution into erythrocytes. Whole blood is often the only specimen available in forensic investigations. However, cannabinoid administration under controlled conditions provide scientific data for interpretation of cannabinoid tests but frequently report plasma concentrations. Cannabinoid ratios for whole-blood/plasma from simultaneously collected authentic specimens are rarely reported. Whole blood was collected for 7 days from 32 individuals residing in a closed research environment. Plasma was produced from part of the whole blood. Both whole blood and plasma were stored at -20°C until analysis by validated 2-dimensional GC-MS methods. Whole-blood/plasma cannabinoid ratios were analysed in 187 specimen pairs. Median (interquartile range) whole-blood/plasma ratios were 0.39 (0.28-0.48)

for THC (n = 75), 0.56 (0.43-0.73) for 11-OH-THC (n = 17), and 0.37 (0.24-0.56) for THCCOOH (n = 187). Intrasubject variability was determined for the first time: 18.1%-56.6% CV (THC) and 10.8%-38.2% CV (THCCOOH). The mean whole-blood/plasma THC ratio was significantly lower than the THCCOOH ratio (P = 0.0001; 4 participants' mean THCCOOH ratios were >0.8). Intra- and intersubject whole-blood/plasma THC and THCCOOH ratios will assist in interpretation of whole-blood cannabinoid data

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J Anal Toxicol 2009 **33** (4) 185

Identifying new cannabis use with urine creatine-normalized THCCOOH concentrations and time intervals between specimen collections

The detection of new cannabis use with creatinine-normalized 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) in urine by a previously recommended procedure for treatment, workplace and judicial drug testing applications was refined in respect of consideration of the time interval between urine collections. Urine specimens were obtained from six less-than- daily cannabis users who smoked placebo, 1.75%, and 3.55% THC cigarettes in randomized order with each separated by one week. Ratios (n = 24,322) were derived by dividing each creatinine-normalized THCCOOH concentration (U2) by that of a previously collected specimen (U1). Maximum, 95% limit, and median U2/U1 ratios with 15 and 6 ng THCCOOH/ml cutoff concentrations, with and without new use between specimens, were obtained for each 24-h interval after smoking up to 168 h and included in tables. The ratios declined with increasing period between collections facilitating improved decision values for determining new cannabis use. For 15 ng THCCOOH/ml, the cutoff concentration and no new use between specimens, the maximum, 95% limit, and median U2/U1 ratios were 3.05, 1.59, and 0.686, respectively, when the collection interval was < or = 24 h and 0.215, 0.135, and 0.085 when it was 96-119.9 h

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Xenobiotica 2009 **39** (5) 391

***In vitro* stability and metabolism of salvinorin A in rat plasma**

Recently, *Salvia divinorum*, a Mexican plant which contains salvinorin A, a hallucinogen has been widely available. The degradtion of salvinorin A in rat plasma, the esterases responsible for its metabolism, and the degradation products have been investigated. The apparent first-order rate constants of salvinorin A at 37°C, 25°C, and 4°C were 3.8 x 10^{-1}, 1.1 x 10^{-1}, and < 6.0 x 10^{-3}/h, respectively. The metabolism of salvinorin A was markedly decreased following the addition of sodium fluoride, an esterase inhibitor. Furthermore, phenylmethylsulfonyl fluoride (serine esterase inhibitor) and bis-*p*-nitrophenyl phosphate (carboxylesterase inhibitor) also reduced salvinorin A catabolism. However, little or no suppression of the degradation was observed with 5,5-dithiobis-2-nitrobenzoic acid (arylesterase inhibitor), ethopropazine (butyrylcholinesterase inhibitor), and BW284c51 (acetylcholineseterase inhibitor). The results suggested that carboxylesterase was primarily involved in the salvinorin A hydrolysis in rat plasma. Catabolism of salvinorin A investigated by liquid chromatography-mass spectrometry included the deacetylated form (salvinorin B) and the lactone-ring-open forms of salvinorin A and salvinorin B. This indicates that lactone-ring-opening reactions were involved in calcium-dependent lactonase

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Chromatographia 2009 **69** (9-10) 933

MAE-GC determination of methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in human urine

Human urine was analysed by using microwave-assisted extraction (MAE) followed by gas chromatography analysis with flame ionization detection for the presence of methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA). Investigations of the extraction efficiency, experimental parameters on the extraction, including extraction solvent and its volume, pH value of sample, extraction time and temperature were conducted. The average recoveries of MA, MDA and MDMA were 92.25, 85.94 and 91.50% respectively, under the optimal conditions. The intra-day and inter-day relative standard deviations were less than 6.9%. The data suggest that the developed procedure is rapid, accurate and sensitive, and may be employed for the simultaneous determination of MA, MDA and MDMA in urine for forensic applications

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Forensic Sci Int 2009 **188** (1-3) 131

Determination of the metabolites of the new designer drugs bk-MBDB and bk-MDEA in human urine

Two new designer drugs 2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one (bk-MBDB) and 2-ethylamino-1-(3,4-methylenedioxyphenyl)propan-1-one (bk-MDEA) were investigated in respect of their metabolites in human urine by employing synthesized standards. *N*-dealkylation, demethylenation followed by *O*-methylation, and β-ketone reduction were identified as the major metabolic pathways GC/MS and LC/MS data. Quantitation by LC/MS demonstrated that both demethylenation followed by *O*-methylation and β-ketone reduction were superior to *N*-dealkylation and that both bk-MBDB and bk-MDEA were generally metabolized into their corresponding 4-hydroxy-3-methoxy metabolites (4-OH-3MeO metabolites). Following hydrolysis, the concentrations of 4-OH-3MeO metabolites and 3-hydroxy-4-methoxy metabolites of both bk-MBDB and bk-MDEA increased dramatically, providing evidence that the metabolites mainly exist as their conjugates

11 Narcotics

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Forensic Sci Int 2009 **186** (1-3) 36

Simultaneous screening and quantification of 25 opioid drugs in post-mortem blood and urine by liquid chromatography-tandem mass spectrometry

The simultaneous screening and quantification of post-mortem blood and urine samples by LC-MS/MS is described for the fentanyls alfentanil, fentanyl, *p*-fluorofentanyl, *cis*-3-methylfentanyl, *trans*-3-methylfentanyl, α-methylfentanyl, norfentanyl, remifentanil, sufentanil, and the other opioid drugs 6-acetylmorphine, buprenorphine, codeine, dextropropoxyphene, ethylmorphine, heroin, methadone, morphine, naloxone, naltrexone, norbuprenorphine, normethadone, oxycodone, pentazocine, pethidine, and tramadol. Extraction of samples was achieved with butyl acetate at pH 7. The drugs were separated by LC on a Genesis C_{18} reversed-phase column with a gradient composed of acetonitrile and ammonium acetate at pH 3.2. Mass spectrometric analysis was carried out with a quadrupole-linear ion-trap mass spectrometer equipped with a turbo ion spray interface in positive mode using multiple reaction monitoring (MRM). Quantification employed five isotope-labelled internal standards. Validation included assessment of linearity, limit of quantification, inaccuracy, precision, and matrix effects. The limits of quantification were sufficient for screening and quantification of opioid drugs at low therapeutic or abuse concentration levels with inaccuracy less than 23% and precision greater than 24% both in blood and urine samples. When applied to autopsy cases, the results of this method were in agreement with those of reference methods

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Forensic Sci Int 2009 **187** (1-3) 34

A validated hybrid quadrupole linear ion-trap LC-MS method for the analysis of morphine and morphine glucuronides applied to opiate deaths

A procedure has been developed which can accurately measure morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in plasma, whole blood and post-mortem blood by employing a hybrid quadrupole linear ion-trap mass spectrometer using an electrospray ionisation ion source coupled to a HPLC system following solid-phase extraction. Other opioids and related compounds such as codeine, dihydrocodeine (and metabolites), noscapine, papaverine and 6-acetylmorphine (6-AM) may be identified. The procedure has been successfully compared with an existing laboratory method using a now discontinued radio-immunoassay technique. In addition, the benefit of measuring the glucuronides directly rather than following deconjugation by β-glucuronidase has been demonstrated. By employing deuterated morphine and M3G as internal standards, etection and quantification of compounds was accomplished using multiple reaction monitoring (MRM). Precision and accuracy was judged to be less than 10% at both high and low levels for all analytes and the calibration curve appeared linear over an acceptable range. Recovery from blood was not less than 90% and ion suppression/enhancement was demonstrated to be less than 15%. Over 130 post-mortem cases involving the use of heroin, prescribed morphine and codeine were analysed. The variation in concentrations of morphine, M3G and M6G was great (especially in heroin and prescribed morphine cases), indicating the many different factors involved with therapeutic use or fatal opiate poisonings, such as tolerance associated with regular use, variable dose regimens and co-administration of

other drugs. Other compounds from opium poppy such as noscapine and papaverine and metabolites of diacetylmorphine in the blood (6-AM) facilitated determination of the source of the morphine (i.e. illicit heroin) and the rapidity of death after administration

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Forensic Sci Int 2009 **187** (1-3) 73

Optimization of HS-SPME/GC-MS analysis and its use in the profiling of illicit Ecstasy tablets (Part 1)

The profiling of traces present in 3,4-methylenedioxymethylampethamine (MDMA) was achieved by the development of a headspace solid-phase microextraction procedure (HS-SPME). Following extraction with HS-SPME traces were analyzed by gas chromatography-mass spectroscopy (GC-MS). HS-SPME conditions were optimized. Best results were produced when 40 mg of crushed MDMA sample was heated at 80°C for 15 min, followed by extraction at 80°C for 15 min with a polydimethylsiloxane/divinylbenzene coated fibre. Traces of 31 compounds were identified related to MDMA synthesis. These may be precursors, intermediates or by-products. Also detected were some fatty acids used as tabletting materials and caffeine used as adulterant. A proposal is made to employ of a restricted set of 10 target compounds for developing a screening tool for clustering samples having close profile. Analysis of 114 seizures was made using an SPME auto-sampler (MultiPurpose Samples MPS2), purchased from Gerstel GMBH & Co. (Germany), and coupled to GC-MS. Data were handled with various pre-treatment methods, followed by the study of similarities between sample pairs based on the Pearson correlation. The data indicate that HS-SPME coupled with a suitable statistical method is a powerful tool for distinguishing specimens coming from the same or different seizures. This information may be employed by law enforcement authorities to visualize the Ecstasy distribution network as well as the clandestine tablet manufacturing

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Forensic Sci Int 2009 **188** (1-3) 144

Simultaneous quantification of buprenorphine, norbuprenorphine, buprenorphine-glucuronide and norbuprenorphine-glucuronide in human umbilical cord by liquid chromatography tandem mass spectrometry

The simultaneous determination of buprenorphine (BUP), norbuprenorphine (NBUP), buprenorphine-glucuronide (BUP-Gluc) and norbuprenorphine-glucuronide (NBUP-Gluc) in human umbilical cord was achieved with LCMS which was both developed and validated. Quantification was accomplished by selected ion monitoring of precursor ions *m/z* 468.4 for BUP; 414.3 for NBUP; 644.4 for BUP-Gluc and 590 for NBUP-Gluc. BUP and NBUP were identified by MS^2, with *m/z* 396, 414 and 426 for BUP, and *m/z* 340, 364 and 382 for NBUP. Glucuronide conjugates were analysed with MS^3 using *m/z* 396 and 414 for BUP-Gluc and *m/z* 340 and 382 for NBUP-Gluc. The assay was linear 1-50 ng/g. Intra-day, inter-day and total assay imprecision (%RSD) were <14.5%, and analytical recovery ranged from 94.1% to 112.3% for all analytes. Extraction efficiencies were >66.3%, and process efficiency >73.4%. In absolute value, matrix effect ranged from 3.7% to 7.4% (CV<21.8%, $n = 8$). The technique was selective without endogenous or exogenous interferences from 41 compounds evaluated. Sensitivity was high with limits of detection of 0.8 ng/g. To test the method, it was applied to an authentic umbilical cord obtained from an opioid-dependent pregnant woman receiving BUP. BUP was not detected but concentrations of the other metabolites were NBUP-Gluc 13.4 ng/g, BUP-Gluc 3.5 ng/g and NBUP 1.2 ng/g

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Forensic Sci Int 2009 **188** (1-3) 23

Microwave-assisted extraction in toxicological screening of skeletal tissues

A technique for the screening of decomposed bone tissue for model drugs of abuse using microwave-assisted extraction (MAE) has been developed. Rats received 50 mg/kg (i.p.) pentobarbital ($n = 2$), 75 mg/kg (i.p.) ketamine ($n = 2$) or 16 mg/kg (i.p.) diazepam ($n = 1$), or remained drug-free (control). Animals were euthanized within 20 min of drug administration and remains were allowed to decompose in a secure outdoor environment to the point of complete skeletonization. Bones (tibiae, femora, vertebrae, ribs, pelvi, humeri and scapulae) were collected and pooled (according to drug) in order to mitigate inter-bone differences in drug distribution. Bones were crushed and cleaned of marrow and residual soft tissue in alkaline solution or phosphate buffer with ultrasonication. Cleaned bones were ground and subjected to MAE in phosphate buffer (pH 6), methanol or a methanol:water mixture (1:1, v/v) at atmospheric pressure in a domestic microwave oven, or passive extraction in methanol. Bone extracts (control and drug-exposed) containing methanol were evaporated to dryness before reconstitution in the phosphate buffer. Bone extracts in phosphate buffer were assayed with ELISA. Absorbance values were expressed as the decrease in absorbance, measured as a percentage, relative to the corresponding drug-free control bone extract. The semi-quantitative nature of the ELISA assay permitted investigation of the effects of extraction solvent and bone sample mass on the assay response for each drug examined, and subsequently comparison with assays of extracts produced by passive methanolic extraction of various bone tissues. In general, the time required for maximal extraction varied with extraction solvent and bone mass for each drug investigated. However, there was significant extraction for all solvent systems employed. MAE appears to represent a significantly faster extraction system compared with passive extraction. Marked extraction recovery was noted within 1 min of exposure for all samples examined. The implications of the data within the context of the available literature on drug analysis in skeletal tissues are discussed

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J Anal Toxicol 2009 **33** (5) 278

Quantitative analysis of carisoprodol and meprobamate in whole blood using benzylcarbamate and deuterated meprobamate as internal standards

In impaired driving casework, two frequently encountered drugs are carisoprodol and meprobamate. Until recently, deuterated internal standards, although desirable, were not available. Previous published studies report the use of a variety of non-deuterated internal standards. However, many do not possess the chemical and physical similarities required for quantitative analysis. By employing benzylcarbamate and meprobamate-d_7 as internal standards, carisoprodol and meprobamate were determined in whole blood by solid-phase extraction and gas chromatography-mass spectrometry. When benzylcarbamate was employed as internal standard, the linear ranges for carisoprodol and meprobamate were 0-20 mg/l and 0-40 mg/l, respectively. However, the linear range increased to 100 mg/l when meprobamate-d_7 was used. Limits of detection for carisoprodol and meprobamate were 0.2 and 0.4 mg/l, respectively, irrespective of the internal standard selection. The limit of quantitation for both drugs using either internal standard was 0.4 mg/l. Accuracies using benzylcarbamate and meprobamate-d_7 were 100-106% and 91-100%, respectively. Corresponding values for precision produced intra-assay coefficients of variation of 2.6-4.3% for benzylcarbamate and 1.0-2.3% for meprobamate-d_7. No carryover was evident at 100 mg/l, the highest concentration tested, and no interferences were noted. Results suggest that either benzylcarbamate or meprobamate-d_7 is a suitable internal standard for quantitative determination of carisoprodol or meprobamate from whole blood

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J Anal Toxicol 2009 **33** (3) 121

Concentration of oxymorphone in postmortem fluids and tissue

The semi-synthetic opioid analgesic, oxymorphone, has been available as Numorphan since the 1950s in the form of injectable solutions and a suppository. More recently, in 2006, oxymorphone was approved by the Federal Drug Administration for use in the form of immediate and extended release tablets under the trade name Opana. Following the introduction of Opana, the number of deaths involving oxymorphone has risen considerably in the State of North Carolina. However, to date in the literature, there are very few reported values for postmortem concentrations of oxymorphone to aid in the interpretation of these cases. Thirty-three medical examiner cases involving oxymorphone are described with the distribution of oxymorphone in postmortem blood, liver, and urine samples. Oxymorphone was identified in blood by enzyme immunoassay and confirmed by gas chromatography-mass spectrometry employing a solid-phase extraction procedure. Calibration curves from 0.025 to 0.50 mg/l were derived with a limit of quantitiation of 0.025 mg/l. In postmortem central ($n = 28$) and peripheral ($n = 23$) blood samples, the mean concentration for oxymorphone was 0.15 mg/l. The median values for the central and peripheral samples were 0.10 mg/l (range: 0.011-0.59) and 0.075 mg/l (range: 0.017-0.82), respectively. Concentrations in the liver ranged from "none detected" to > 2.0 mg/kg, with mean and median values of 0.36 and 0.30 mg/kg, respectively. The majority of urine samples were > 0.50 mg/l

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Appl Spectrosc 2009 **63** (5) 507

Some advances in Fourier transform infrared transflection analysis and potential applications in forensic chemistry

Qualitative and quantitative analysis in the mid-infrared region is facilitated by the transflection technique. The lower absorbance values typically encountered in the near-infrared region is mitigated by the higher sensitivity for bands in the mid-infrared. Small sample size and little sample preparation facilitate analysis of forensic substances. Even with little or no sample preparation, small amounts of illicit drugs such as cocaine may be reliably and nondestructively identified. By employing the transflection technique described in this paper, single grains may be rapidly identified simply by placing the sample on a specially designed substrate and recording the Fourier transform infrared (FT-IR) spectrum. Transflection was employed in the qualitative analysis of aqueous solutions and solid particles using relatively simple equipment in conjunction with a commercially available diffuse reflection accessory. Improvements in both equipment and technique are identified. Extensions of the equipment into two new forms, with potential uses in proteomics and forensics, are described

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Appl Spectrosc 2009 **63** (6) 689

Studying the effect of pH variation on the incorporation of the antipsychotic drug clozapine into dyed and non-dyed hair samples using micro-attenuated total reflection spectroscopy

Among the mostly widely used medications are antipsychotic drugs. Furthermore, they are frequently taken for prolonged periods. Hair provides a useful indication of long-term exposure due to its accumulation and trapping of the drugs. Possible changes in the structural components of hair occur as a result of the drug exposure are also of interest. Micro-attenuated total reflection (ATR) spectroscopy facilitates the investigation of the structural changes of hair samples by the application of sufficient pressure and without microtoming the hair [A. Kocak and S. L. Berets, *Appl. Spectrosc*. **62**, 803 (2008)]. Changes resulting from exposure of dyed and undyed hair to external clozapine as a function of the pH of the exposing solution were determined. Single samples from different individuals and in one case from different areas of the scalp of the same individual were analyzed. The data confirm that pH related differences exist between drug-exposed dyed and non-dyed hair samples

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Anal Bioanal Chem 2009 **394** (4) 1161

Automated on-line in-tube solid-phase microextraction coupled with HPLC/MS/MS for the determination of butyrophenone derivatives in human plasma

The analysis of six butyrophenone derivatives (moperone, floropipamide, haloperidol, spiroperidol, bromperidol, and pimozide) in human plasma samples was achieved using a fully automated on-line method combining in-tube solid-phase microextraction (SPME) in which sample clean-up and enrichment are conducted through an open tubular fused-silica capillary column and high-performance liquid chromatography (HPLC)/tandem mass spectrometry (MS/MS) detection. The six butyrophenones were extracted by repeatedly aspirating and dispensing plasma sample solutions on a DB-17 capillary column (60 cm x 0.32 mm i.d., film thickness 0.25 µm). Analytes adsorbed on the inner surface of the capillary column were eluted with acetonitrile-rich mobile phase using a gradient separation technique. Extraction efficiencies ranged from 12.7% to 31.8% for moperone, spiroperidol, and pimozide, and from 1.08% to 4.86% for floropipamide, haloperidol, and bromperidol. Regression equations of the six butyrophenones exhibited excellent linearity, ranging from 0.05 to 50 ng/0.1 ml of plasma, except for moperone and spiroperidol (0.01 to 50 ng/0.1 ml).The limits of detection and quantification in plasma for each butyrophenone were 0.03-0.2 and 0.1-0.5 ng/ml, respectively. The intra- and inter-day coefficients of variation for all drugs in plasma were less than 13.7%

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Forensic Sci Int 2009 **187** (1-3) 14

Blood-positive illicit-drug findings: Implications for cause-of-death certification, classification and coding

Cause-of-death data are required for national health administration, international comparisons and epidemiological research. The compilation of mortality statistics begins with investigation of causes of death, proceeds with medical death certification and concludes with coding of causes and selection of the underlying cause of death at the statistical registry. The aim of the current investigation was to assess how unequivocally and specifically the toxicologically verified intoxication of cannabinoids, opiates, amphetamines and cocaine is represented in diagnostic entries on death certificates, in the national cause-of-death database and ultimately, in the cause-of-death statistics. Deaths with blood-positive drug finding(s), the corresponding death certificates and the information entered in Statistics Finland's cause-of-death database were analysed for the entire years of 2000, 2002 and 2004. Drug presence at the time of death may or may not be related to death. A drug-related problem was found to be the cause of death in 52% of cannabinoid-positive, in 81% of amphetamine-positive, in 98% of opiate-positive and in 100% of cocaine-positive deaths, calculated from the combined three-year material. By employing the cause-of-death database, specific drug-related diagnosis could be identified in 21% of cannabinoid-positive, in 89% of opiate-positive and in 57% of amphetamine-positive deaths. Corresponding proportions of specific drug-related underlying causes in the cause-of-death statistics were even smaller for cannabinoids and amphetamines at 10% and 39% respectively. In multiple-drug cases, identification was possible only if each drug had been assigned an additional drug-specific code from "T categories" of ICD-10 Chapter XIX. Extrordinarily, a third of cannabinoid-related and a quarter amphetamine-related cause-of-death diagnoses had been assigned to unspecific categories of ICD-10 in the multiple-cause database and, more notably, in cause-of-death statistics based on selected underlying causes. It is proposed that a better specification of drug-related causes of death could be achieved if the next ICD revision provided each drug with the code of its own, e.g. one comparable to ATC (Anatomical Therapeutic Chemical Classification Index) codes, to be used for its specification in all positions and combinations, or at least provide compatibility with the ATC's coding system. In addition, for classification and description of the trends of drug-related deaths, equal and specific definitions for drug-related deaths would also be required

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J Anal Toxicol 2009 **33** (3) 174

Relationship between serum glycolate and falsely elevated lactate in severe ethylene glycol poisoning

In respect of ethylene glycol (EG) poisoning, a falsely raised serum lactate concentration has been proposed to be an assay cross-reaction with glycolate. However, a concentration-dependent relationship has not been established. Serum lactate and glycolate concentrations have been investigated in a case of severe EG poisoning. Serial EG [by gas chromatography (GC)], glycolate (derivatized to methyl glycolate, analysis by GC), and lactate (both enzymatic spectrophotometry and GC) concentrations were correlated at five time points. A false-positive lactate assay result was noted in the absence of lactate on GC analysis. The correlation coefficient (Pearson's *r*) between lactate (by enzymatic spectrophotometry) and glycolate was 0.984 and was statistically significant ($p < 0.01$). The mean lactate/glycolate conversion factor was 2.58 +/- 0.95. A linear correlation between falsely elevated serum lactate and glycolate concentrations was identified in a case of severe EG poisoning. The data provide further support to the proposal that the lactate assay may cross-react with glycolate in EG poisoning

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J Anal Toxicol 2009 **33** (4) 212

Determination of olanzapine in whole blood using simple protein precipitation and liquid chromatography-tandem mass spectrometry

The quantification of an antipsychotic drug (olanzapine) in whole blood using dibenzepine as internal standard (IS) was achieved by development of a simple, sensitive, and reproducible liquid chromatography-tandem mass spectrometry method which has been validated. Following acidic methanol-induced protein precipitation of the whole blood samples, olanzapine and IS were chromatographed on a reversed-phase Zorbax Extend-C_{18}-column at pH 9.0. Quantification was achieved with a triple-quadrupole mass spectrometer using electrospray ionization operating in multiple reaction monitoring and positive ion mode. Total chromatographic run time was 15 min, and calibration curve was linear over the concentration range of 0.005 to 0.50 mg/kg olanzapine in whole blood. Validation of the procedure was conducted in respect of selectivity, matrix interference, recovery, linearity, limit of detection, limit of quantitation (LOQ), accuracy, precision, and stability. Absolute recoveries obtained were 103% for olanzapine and 68% for IS. An LOQ of 0.005 mg/kg olanzapine in whole blood was achieved. Inter- and intraday precision were less than 11% within concentrations from 0.01 to 0.50 mg/kg, and the accuracy ranged from 85 to 115%. Subsequently, the technique was applied to 27

authentic samples, of which 20 were postmortem blood samples, from forensic investigations

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J Agric Food Chem 2009 **57** (10) 4058
Quantitative analysis of tetramethylenedisulfotetramine (tetramine) spiked into beverages by liquid chromatography-tandem mass spectrometry with validation by gas chromatography-mass spectrometry
Hundreds of deliberate and accidental food poisoning events in China involve tetramethylenedisulfotetramine, commonly known as tetramine, a highly neurotoxic rodenticide (human oral LD_{50} = 0.1 mg/kg). Quantitation of tetramine spiked into beverages has been investigated, including milk, juice, tea, cola, and water, with cleanup by C_8 solid phase extraction and liquid-liquid extraction. High-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) based upon fragmentation of *m/z* 347 to *m/z* 268 was employed. The procedure was validated by gas chromatography-mass spectrometry (GC-MS) operated in selected ion monitoring mode for ions *m/z* 212, 240, and 360. Limit of quantitation was 0.10 µg/ml by LC-MS/MS *versus* 0.15 µg/ml for GC-MS. Spiking of the beverages at 2.5 and 0.25 µg/ml resulted in recoveries ranging from 73 to 128% by liquid-liquid extraction for GC-MS analysis, from 13 to 96% by SPE, and from 10 to 101% by liquid-liquid extraction for LC-MS/MS analysis

13 Alcohol

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J Anal Toxicol 2009 **33** (3) 155
Ethylglucuronide determination in urine and hair from alcohol withdrawal patients
Two methods have been developed by liquid chromatography-tandem mass spectrometry for the determination of ethylglucuronide (EtG) in urine and in hair. The two methods were fully validated, including linearity (0.25-100 µg/ml in urine; 0.05-5 ng/mg in hair; $r^2 > 0.99$, $n = 5$), limits of detection (0.1 µg/ml in urine, 0.025 ng/mg in hair) and quantitation (lowest level of the calibration curve), extraction efficiency (> 55%), within-day and between-day imprecision and bias (CV and mean relative error < 15%), matrix effect, and relative ion intensity. The methods were applied to 541 urine samples and 17 hair specimens collected from 156 alcohol withdrawal patients. The determination of ethanol *versus* EtG in urine was compared. In addition, the convenience of EtG determination in hair was examined. EtG in urine and in hair was demonstrated to be a powerful tool for monitoring abstinence in these alcohol withdrawal patients

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Forensic Sci Int 2009 **186** (1-3) 52
Assessment of the stability of the ethanol metabolite ethyl sulfate in standardised degradation tests
In addition to the ethanol metabolite, ethyl glucuronide (EtG) which may be prone to biological degradation, ethyl sulfate (EtS), a non-oxidative metabolite of ethanol, has been employed for forensic purposes as an ethanol consumption marker. Ethanol is widely consumed in many western cultures and whereas EtS until now was thought to be resistant to bacterial degradation, knowledge about its stability is of importance for forensic investigations. This was tested using standardized test methods from the panel of OECD tests. The results showed that EtS was stable in the closed bottle test (CBT) (OECD 301 D) but not in the manometric respiratory test (MRT) (OECD 301 F) with higher bacterial density. In respect of forensic investigations the stability of EtS and the possibility of bacterial degradation of EtS should be taken into account when alcohol intake some hours prior to death needs to be ruled out by determination of alcohol consumption markers in putrefied corpses, where ethanol concentration might have been produced post-mortem by fermentation processes

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J Anal Toxicol 2009 **33** (4) 204
Determination of ethylglucuronide in oral fluid by ultra-performance liquid chromatography-tandem mass spectrometry
The determination of ethylglucuronide (EtG) in oral fluid was achieved with an ultra-performance liquid chromatography-tandem mass spectrometry method which has been validated. A Hyper-SEP SAX column was employed for sample clean-up by solid-phase extraction. Negative ionization was conducted in the multiple reaction monitoring mode. Two transitions were monitored for the analyte and one for the internal standard EtG-d_5. The calibration range was 4.4-222 ng/ml. Recovery of EtG ranged from 86 to 99%, and the between-assay precisions ranged from 5 to 9% RSD. The limit of quantification was found to be 4.4 ng/ml. Following a moderate alcohol intake, the concentration of EtG in oral fluid collected 2-14 h varied from 13.3 to 57.7 ng/ml

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J Anal Toxicol 2009 **33** (4) 208
Serum/whole blood concentration ratio for ethylglucuronide and ethyl sulfate
Serum/blood (S/B) concentration ratios for ethyl glucuronide (EtG) and ethyl sulfate (EtS) have not been reported. The aim of this study was to investigate the ratios in specimens from patients at admission to an alcohol rehabilitation clinic. Two blood samples were obtained simultaneously, and EtG and EtS were analyzed in whole blood and serum, respectively, using a liquid chromatography-mass spectrometry procedure. Separate calibration standards were prepared in both whole blood and serum. Analysis was performed on 13 pairs of serum and whole blood. The median S/B value for EtG was 1.69, and the range was 1.33-1.90. For EtS, the median S/B ratio was 1.30, and the range was 1.08-1.47. The S/B ratio was significantly lower for EtS than for EtG ($p < 0.001$). The greater concentrations of EtG and EtS in serum compared with whole blood require consideration when whole blood results obtained from forensic toxicology are compared with serum or plasma results from clinical laboratories

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Forensic Sci Int 2009 **186** (1-3) 56
Five-year update on the occurrence of alcohol and other drugs in blood samples from drivers killed in road-traffic crashes in Sweden
The Swedish National Road Administration (Vagverket) recorded a total of 1403 drivers killed in road-traffic crashes in Sweden between 2003 and 2007. Forensic autopsies were carried out in approximately 97% of all deaths and specimens of blood and urine were sent for toxicological analysis. In 60% of cases (n = 835), toxicology results were negative and 83% of these victims were men. The blood-alcohol concentration (BAC) was above the legal limit for driving (>0.2g/l) in 22% of cases (n = 315) at mean, median and highest concentrations of 1.7 g/l, 1.7 g/l and 4.9 g/l, respectively. The percentages of male to female drivers with BAC>0.2 g/l were 93% *vs* 7% compared with 83% *vs* 17% for those with drugs other than alcohol in blood. Drivers with an illegal BAC were greater in single vehicle crashes compared with multiple vehicle crashes (67% *vs* 33%). The opposite was found for drivers who had taken a prescription drug (39% *vs* 61%) and also for drug-negative cases (31% *vs* 69%). Drugs other than alcohol were identified in 253 cases (18%); illicit drugs only in 39 cases (2.8%), both licit and illicit in 28 cases (2.0%) and in 186 cases (13.3%) one or more therapeutic drugs were present. Amphetamine was the most common illicit drug identified at mean, median and highest concentrations of 1.5 mg/l, 1.1 mg/l and 5.0 mg/l, respectively (n = 39). Blood specimens contained a wide spectrum of pharmaceutical products (mean 2.4 drugs/person), comprising sedative-hypnotics (n = 93), opiates/opioids (n = 69) as well non-scheduled substances, such as paracetamol (n = 78) and antidepressants (n = 93). However, the concentrations of these compounds in blood were mostly in the therapeutic range. Alcohol still remains the psychoactive substance most frequently identified in the blood of drivers killed in road-traffic crashes, despite an appreciable increase (12-fold) in number of arrests made by the police for drug-impaired driving after a zero-tolerance law was introduced (July 1999)

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Forensic Sci Int 2009 **188** (1-3) 40
Age and gender differences in blood-alcohol concentration in apprehended drivers in relation to the amounts of alcohol consumed

Data on people apprehended in Sweden for driving under the influence of alcohol (DUIA) over an 8-year period (2000-2007) were analysed in respect of age, gender, and blood-alcohol concentration (BAC). Duplicate analysis of ethanol was performed in venous blood by headspace gas chromatography and results were reported positive at a cut-off concentration of 0.1 g/l (10 mg/100 ml or 0.01 g%). The mean, median and highest BAC was 1.74 g/l, 1.70 g/l and 5.18 g/l, respectively. Most of offenders were men (89.5%) with a mean age of 39.0+/-14.6 y (+/-SD). Women (10.5%) were several years older at 41.8+/-13.6 y ($p<0.001$). The mean BAC in the men (1.73+/-0.85 g/l) was not significantly ($p>0.05$) different from women (1.77+/-0.87 g/l). The youngest offenders aged 15-20 y ($n = 3513$) had a mean BAC of 1.30+/-0.60 g/l (median 1.32), which was significantly less ($p<0.001$) than people aged 40-50 y (n = 6644); mean 1.90 g/l (median 2.0 g/l). In 95 individuals (89 men and 6 women) the BAC exceeded 4.0 g/l, which is a level required to cause fatality by acute alcohol poisoning. The Widmark formula was employed to calculate that a man (80 kg) with a BAC of 1.7 g/l has 95 g ethanol (approximately 12 units of alcohol) in the body compared with 61 g (approximately 8 units) for a woman (60 kg). The data demonstrate that the average drunken driver in Sweden is typically a binge drinker and education programs and treatment for alcohol-use disorder might be more appropriate compared with the more conventional penalties for alcohol-impaired driving

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Anal Bioanal Chem 2009 **394** (7) 1895

Determination of ethyl-glucuronide in hair for heavy drinking detection using liquid chromatography-tandem mass spectrometry following solid-phase extraction

In respect of monitoring alcohol abuse in both clinical and forensic contexts, the detection of ethyl-β-D-6-glucuronide (EtG), a stable phase II metabolite of ethanol, is of great interest. The detection and quantification of EtG in hair was performed by a liquid chromatography-electrospray ionisation-tandem mass spectrometry technique. Thirty milligrams of washed and cut hair were cleaned using solid-phase extraction graphite cartridges. An Uptisphere-3SI column was employed for separation. Detection was performed in the negative mode. Following validation, the technique was applied to hair samples obtained from four fatalities (F) with documented excessive drinking habits, 12 heavy drinkers (HD) and seven social drinkers (SD). The technique provides limits of detection and quantification of 4 and 10 pg/mg, respectively. Intra- and inter-assay standard deviation and relative bias were less than 20% over the calibrating range (10 to 3,000 pg/mg). EtG hair concentrations in SD were <10 pg/mg and >50 pg/mg for F and HD (range, 54 to 497 pg/mg). Only a very small quantity of hair is required to determine an effective heavy alcohol consumption (EtG hair concentration >50 pg/mg)

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J Anal Toxicol 2009 **33** (3) 167

Potential effect of alcohol content in energy drinks on breath alcohol testing

Positive breath alcohol test results have been claimed by some defendants after the ingestion of non-alcoholic energy drinks in the USA. A number of energy drinks were analysed by gas chromatography and some 88.9% (24 of 27) were found to contain low concentrations of ethanol (5-230 mg/dl). In addition, non-alcoholic energy drinks were consumed (24.6-32 oz) by volunteers to investigate alcohol concentrations on a portable breath-testing instrument. Eleven of 27 (40.7%) beverages gave positive results on a portable breath-testing instrument (0.006-0.015 g/210 l) when samples were taken within 1 min of the end of drinking. All tests taken by portable breath test, DataMaster, and Intox EC/IR II at least 15 min after the end of drinking produced alcohol-free readings (0.000 g/210 l). Allowing subjects a minimum 15-min observation period before breath-alcohol testing eliminates the possibility of a small false-positive alcohol reading

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Forensic Sci Int 2009 **188** (1-3) 140

Comparison of ethyl glucuronide in hair with carbohydrate-deficient transferrin in serum as markers of chronic high levels of alcohol consumption

Ethyl glucuronide in hair (HEtG) and carbohydrate-deficient transferrin (CDT) in serum were investigated both in terms of sensitivity and specificity as markers of heavy drinking. The ethanol daily intake (EDI) during the last 2-week and 3-month periods of 86 volunteers, including teetotalers, social, and heavy drinkers was ascertained by an interviewing technique. HEtG analysis was conducted by a fully authenticated LC-MS-MS procedure and ranged from <LOD (2 pg/mg) to 890.5 pg/mg. CDT was determined by immunonephelometry or by HPLC. Sensitivity and specificity of the two markers as indicators of an EDI higher than 60 g/day were calculated, with cut-off at 27 pg/mg (HEtG) and 2.5% (CDT). In respect of the EDI during the last 2 weeks, HEtG showed equal selectivity (0.93 for both HEtG and CDT-immunonephelometry; 0.70 for both HEtG and CDT-HPLC) and 2 times the sensitivity of either of the two CDT methods (1.00 *vs*. 0.44 for CDT-immunonephelometry; 0.96 *vs*. 0.50 for CDT-HPLC). Considering the EDI of the last 3-months, similar differences in performances but higher absolute sensitivity and selectivity values were noted for HEtG (selectivity: 1.00 for both HEtG and CDT-immunonephelometry, 0.89 and 0.78 for HEtG and CDT-HPLC, respectively; sensitivity: 1.00 *vs*. 0.47 for CDT-immunonephelometry; 0.98 *vs*. 0.51 for CDT-HPLC). The data demonstrate that HEtG when compared with CDT measured by the different methods is a selective marker of ethanol heavy chronic use and provides considerably higher sensitivity

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Anal Chim Acta 2009 **646** (1-2) 128

Headspace solid-phase microextraction-gas chromatography-mass spectrometry determination of the characteristic flavourings menthone, isomenthone, neomenthol and menthol in serum samples with and without enzymatic cleavage to validate post-offence alcohol drinking claims

A procedure has been developed for determination of the flavour compounds menthone, isomenthone, neomenthol and menthol in serum samples with and without enzymatic cleavage. The flavours are characteristic markers for consumption of peppermint liqueurs as well as certain digestif bitters, herbal and bitter liqueurs. The technique involves rapid HS-SPME-GC-MS (headspace solid-phase microextraction-gas chromatography-mass spectrometry). This method facilitated the identification of the four compounds with a limit of detection (LOD) of 2.1 ng/ml (menthone and isomenthone), 2.8 ng/ml (neomenthol) and 4.6 ng/ml (menthol), and a limit of quantification (LOQ) of 3.1 ng/ml (menthone and isomenthone), 4.2 ng/ml (neomenthol) and 6.8 ng/ml (menthol) in serum samples. The technique showed good precision both intraday (3.2-3.8%) and interday (5.8-6.9%) and a calibration curve determination coefficient (r^2) of 0.990-0.996. A volunteer consumed peppermint liqueur on three different days under controlled conditions. At set intervals, blood samples were taken, and the concentration-time profiles for serum menthone, isomenthone, neomenthol and menthol, as free substances as well as glucuronides, were analysed. Both menthol and neomenthol exhibited rapid phase II metabolism but minor amounts of free substances were also detected. Menthone and isomenthone were quickly metabolised and were found in lower concentrations and over a shorter time span than the other analytes. Blood samples taken from 100 drivers who claimed to have consumed peppermint liqueur prior to blood sampling. Menthone, isomenthone, neomenthol and menthol were detected in serum as free substances in concentrations between 3.1 and 7.0 ng/ml in eight cases (menthone), 3.1 and 11.3 ng/ml in eight cases (isomenthone), 5.3 and 57.8 ng/ml in nine cases (neomenthol) and 8.0 and 92.1 ng/ml in nine cases (menthol). The sum values of free and conjugated substances ranged between 4.2 and 127.8 ng/ml in 35 cases for neomenthol and 11.0 and 638.2 ng/ml in 59 cases for menthol. Menthone and isomenthone were not conjugated. The data confirm that the analysis of typical beverage aroma compounds, such as menthone, isomenthone, neomenthol and menthol may be used for specific verification of post-offence alcohol consumption claims

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J Anal Toxicol 2009 **33** (3) 182

Blood ethanol concentrations are less stable than serum or plasma upon storage because of oxyhemoglobin-mediated oxidation of ethanol to acetaldehyde (Letter)

Re: "Comparison among plasma, serum and whole blood ethanol concentrations: Impact of storage conditions and collection tubes. Penetar *et al. J Anal Toxicol.* 2008 **32** (7) 505". The authors of are complimented for their comprehensive and interesting study. However, it is noted that the storage conditions of blood, plasma, and serum ethanol concentrations were not considered in the study. In whole blood samples, this has possible implications for the temperature-dependent oxidation of ethanol to acetaldehyde by oxyhemoglobin which is not prevented by the addition of sodium fluoride. Catabolism of ethanol by

oxidation is independent of concentration and is enhanced increasing temperature. When stored at 4°C, the mean ethanol loss has been found to be 0.02 mg/dl/day, and this increased to 0.29 mg/dl/day at 22°C and to 6.0 mg/dl/day at 37°C. Consequently, under the most extreme storage condition used by Penetar *et al*. (i.e., 10 days storage at 25°C), whole blood ethanol concentration would be at least 3 mg/dl lower because of ethanol oxidation by oxyhemoglobin when compared with serum and plasma samples. However, this difference may not be evident as the interassay CV of the method used in this study was 5.9% at an aqueous ethanol standard of 100 mg/dl

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J Anal Toxicol 2009 **33** (3) 183

Blood ethanol concentrations are less stable than serum or plasma upon storage because of oxyhemoglobin-mediated oxidation of ethanol to acetaldehyde (Reply)

No abstract available

14 Tobacco

Hegstad S, Stray-Pedersen A, Olsen L, Vege A, Rognum TO, Morland J, Christophersen AS// Norwegian Inst Publ Hlth, Division Forensic Toxicol & Drug Abuse, POB 4404, NO-0403 Oslo, Norway

J Anal Toxicol 2009 **33** (4) 218

Determination of cotinine in pericardial fluid and whole blood by liquid chromatography-tandem mass spectrometry

The main metabolite of nicotine is cotinine. Therefore, it is employed as an indicator of exposure to tobacco smoke. The quantification of cotinine in pericardial fluid and whole blood collected from autopsy casework involving cases of infant death has been investigated by the development of a suitable technique. Sample clean-up was achieved by solid-phase extraction with a mixed-mode column. Liquid chromatography-tandem mass spectrometry was employed to quantify cotinine. Positive ionization was performed in the multiple reaction monitoring mode. Two transitions were monitored for the analyte and one for the internal standard, cotinine-d_3. In both matrices, the calibration range was 0.9-176 ng/ml for cotinine. Recovery of the analyte ranged from 86 to 92%, and the between-assay precisions ranged from 4 to 6% relative standard deviation. During autopsy, whole blood and pericardial fluid samples from 95 infant deaths were obtained and analysed. A strong correlation (r^2 = 0.97) was recorded between the cotinine concentrations in pericardial fluid and blood. Postmortem time interval did not affect the correlation. Therefore, in forensic autopsies, pericardial fluid may be an alternative specimen to blood for quantification of cotinine

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J Chromatogr B 2009 **877** (11-12) 1185

Simultaneous determination of four tobacco-specific *N*-nitrosamines (TSNA) in human urine

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-nitrosonornicotine (NNN), *N*-nitrosoanabasine (NAB) and *N*-nitrosoanatabine (NAT) are tobacco-specific *N*-nitrosamines (TSNA). They have been proposed to be involved in tobacco smoke carcinogenesis. An LC-MS/MS procedure was developed and validated for the determination of total (free and conjugated) TSNA in human urine. Limits of detection (LOD) were 2.0, 0.8, 1.1 and 0.7 pg/ml for NNAL, NNN, NAB and NAT, respectively. Significantly higher levels of TSNA was found in smokers urine compared with nonsmokers. The newly developed procedure is suitable for investigating tobacco use-related exposure to NNK, NNN, NAB and NAT

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J Pharm Biomed Anal 2009 **49** (5) 1165

Validation of a GC-FID method for rapid quantification of nicotine in fermented extracts prepared from *Nicotiana tabacum* fresh leaves and studies of nicotine metabolites

The rapid and reliable quantitation of nicotine in tobacco leaf extracts was examined with a new GC-FID technique which was both developed and validated. An amine-deactivated capillary column was employed to prevent nicotine adsorption on the column. The procedure was employed to study the degradation of nicotine in a fermented aqueous extract. A loss of nearly 20% of nicotine over 12 months was noted. Close examination of GC-MS runs from concentrated samples of the same extract demonstrated the presence of nicotine metabolites such as nornicotine, anatabine, myosmine, 2,3'-bipyridyl, and 2-pyrrolidinone

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J Chromatogr B 2009 **877** (14-15) 1575

A modified method for the determination of tobacco specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in human urine by solid phase extraction using a molecularly imprinted polymer and liquid chromatography tandem mass spectrometry

A previously published method for the determination of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine by solid phase extraction on a molecularly imprinted polymer column coupled with HPLC and -MS/MS detection was further developed. The influence of ion suppression due to sample matrix effect was investigated and found to influence the response of NNAL. By modifying the liquid chromatography conditions, the response for this method was increased approximately 25-fold by avoidance of ionization suppression that was found with a previously published method and sample throughput was improved. The dynamic range of the assay extended from 20 to 2500 pg/ml with a mean r^2>0.998. The lower limit of quantitation for the assay was 20 pg/ml in spite of the use of an instrument with inherently lower sensitivity. The procedure was authenticated in respect of current FDA guidelines for bioanalytical method validations

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J Anal Toxicol 2009 **33** (5) 260

Quantitation of *N*'-nitrosonornicotine (NNN) in smokers' urine by liquid chromatography-tandem mass spectrometry

Tobacco-specific nitrosamine *N*'-nitrosonornicotine (NNN) is carcinogenic to humans (IARC Group 1). Quantitation of tobacco smoke-related exposure to NNN with suitable biomarkers is of interest for risk analysis. NNN and NNN-*N*-glucuronide have been quantified in urine of smokers recently. However, what percentage of the absorbed dose of NNN is excreted as total NNN (sum of free and conjugated NNN) in urine of smokers is not available. A sensitive procedure based on liquid chromatography with tandem mass spectrometry with deuterium-labeled internal standard for the determination of total NNN in human urine has been developed. The limit of quantitation of the technique was 2 pg/ml with a calibration line linear up to 256 pg/ml. In 16 smokers, the respiratory retention of NNN was measured through controlled smoking. It was found that on average about 1% of the pulmonary NNN dose was excreted in 24 h urine as total NNN

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J Pharm Biomed Anal 2009 **49** (5) 1256

The enzyme-linked immunosorbent assay (ELISA) method for nicotine metabolites determination in biological fluids

Enzyme-linked immunosorbent assay (ELISA) was employed in the determination of nicotine metabolites in urine and serum samples from active smokers and the results compared with thin layer chromatography (TLC) and densitometry. Specific anti-cotinine antibodies were produced from rabbit sera after sequential immunization with 4'-carboxycotinine-hemocyanine conjugate. Immunoaffinity chromatography with self-prepared cotinine-aninohexyl-sepharose bed facilitated the isolation of the specific anti-nicotine metabolites antibodies from the antiserum. Antibodies to cotinine were passively immobilised on ELISA plates for competition studies between nicotine metabolites in samples and tracer (horseradish peroxidase-cotinine conjugate). Following the washing stage, the enzymatic activity of solid-phase-bound peroxidase was measured. For calibration, cotinine perchlorate solutions in an appropriate matrix were employed. Determination ranges for serum and urine samples were from 3 to 1500 and from 3 to 5000 ng/ml, respectively. Precision within-run and between-run was less than 8.7 and 11.3%; mean recovery of cotinine was 100.59% from serum and 88.56% from urine samples. ELISA employed in the determination of the main nicotine metabolites demonstrated high accuracy and sensitivity. However, this method was less specific than the reference technique (TLC). The high correlation coefficients (r>0.9) between the data for nicotine metabolites in urine by means of ELISA and TLC with densitometry validated the possibility for the use of enzyme-linked immunosorbent assay for practical monitoring of tobacco smoke exposure in large population groups

15 Homeland Security

Alava T, Berthet-Duroure N, Ayela C, Trevisiol E, Pugniere M, Morel Y, Rameil P, Nicu L*// *LAAS-CNRS, 7 Ave Colonel Roche, FR-31077 Toulouse, France

Sensor Actuator B Chem 2009 **138** (2) 532

Parallel acoustic detection of biological warfare agents surrogates by means of piezoelectric immunochips

Four different biological warfare agents (BWA) surrogates were analysed using flow functionalization of piezoelectric immunochips. To conduct parallel analysis of all BWA surrogates simultaneously, the E4 Quartz Crystal Microbalance with Dissipation monitoring system (QCM-D) was employed. Firstly, examination of antibodies immobilization, parallel detection of related BWA surrogates diluted in buffer solutions and regeneration of the complex antibodies/BWA surrogates are considered. Minimal detection thresholds for *Escherichia coli* MRE 162, *Bacillus atrophaeus*, *Cydia pomonella* granulosis virus (CpGV) and ovalbumin are respectively equal to 2.4 × 107 CFU/ml, 1.4 × 106 spores/ml, 1.1 × 108 granules/ml and 1 µg/ml. Detection experiments for three of the four BWA surrogates (*E. coli* MRE 162, *B. atrophaeus* and ovalbumin) immersed in real liquid matrices from air sampler were achieved successfully

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Anal Chem 2009 **81** (14) 5637

Functional magnetic nanoparticles for biodefense and biological threat monitoring and surveillance

Biodefense and threat surveillance by water-dispersible, bactericidal, paramagnetic nanoparticles by a new procedure is presented. The nanoparticles consist of magnetite clusters (~70 nm) modified by polyethylene-imine (PEI) and poly(hexamethylene biguanide) (PH-MBG) and are prepared by a two-step economical procedure. The cationic nanoparticles are colloidally stable in water at pH = 10, where they sequester DNA or whole microbes. Subsequently, the nanoparticles and bound DNA are captured by high-gradient magnetic separation. Methods are produced for the efficient extraction and quantification of the DNA by real-time PCR. At concentrations far below cytotoxicity levels for mammalian cells, broad bactericidal activity of the nanoparticles is apparent. The levels of the DNA detection sensitivity produced in our research demonstrate the applicability of the developed method

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Anal Chem 2009 **81** (14) 5935

Femtomolar detection of the anthrax edema factor in human and animal plasma

A toxin which contributes to cutaneous and systemic anthrax is edema factor (EF), a calmodulin-activated adenylyl cyclase. A novel, sensitive assay to monitor functional EF in human and animal plasma has been developed for detection of anthrax toxins in humans or animals infected by *Bacillus anthracis*. Samples containing EF are incubated in the presence of calmodulin and ATP, which is metabolised to cAMP. Following oxidation and derivatization, cAMP is monitored by competitive enzyme immunoassay. The high turnover of EF and the sensitivity of cAMP detection facilitates EF detection at concentrations of 1 pg/ml (10 fM) in 4 h in plasma from humans or at 10 pg/ml in the plasma of various animal species using only a blood volume of 5 µl. Good reproducibility of the assay was noted with intra- and interday coefficients of variation in the range of 20%. In addition, it was not subject to significant interindividual matrix effects. An experimental study was performed in mice infected with the Berne strain where EF was detected in serum and ear tissues. This simple and robust combination of enzymatic reaction and enzyme immunoassay for the diagnosis of anthrax toxemia might benefit biological threat detection, research and clinical practice

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J Chromatogr A 2009 **1216** (27) 5228

On-line high-performance liquid chromatography-ultraviolet-nuclear magnetic resonance method of the markers of nerve agents for verification of the Chemical Weapons Convention

Alkyl alkylphosphonic acids (AAPAs) and alkylphosphonic acids (APAs) are markers of the toxic nerve agents required for verification of the Chemical Weapons Convention (CWC). An on-flow liquid chromatography-ultraviolet-nuclear magnetic resonance (LC-UV-NMR) method for the retrospective detection and identification of these compounds is described. Firstly, the LC-UV-NMR parameters were optimized for benzyl derivatives of the APAs and AAPAs. Optimization included stationary phase C_{18}, mobile phase methanol:water 78:22 (v/v), UV detection at 268nm and ^{1}H NMR acquisition conditions. The procedure described herein facilitated the detection of analytes through acquisition of high quality NMR spectra from the aqueous solution of the APAs and AAPAs with high concentrations of interfering background chemicals which have been removed by preceding sample preparation. The reported standard deviation for the quantification is related to the UV detector which showed relative standard deviations (RSDs) for quantification within +/-1.1%, while lower limit of detection upto 16 µg (in µg absolute) for the NMR detector. The LC-UV-NMR method was applied to identify the APAs and AAPAs in real water samples following solid phase extraction and derivatization. The procedure is fast (total experiment time approximately 2h), sensitive, rugged and efficient

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Eur J Mass Spectrom 2009 **15** (5) 579

Gas chromatography electron ionization mass spectrometric analysis of O-alkyl methylphosphinates for verification of Chemical Weapons Convention

O-alkyl methylphosphinates (AMPs) are included in schedule 2B4 chemicals in the Chemical Weapons Convention (CWC). Analysis was performed using gas chromatography/mass spectrometric (GC/MS) on a variety of AMPs and their deuterated analogues and revealed that their fragmentations were determined by α-cleavages, McLafferty +1 and hydrogen rearrangements. By employing the electron ionization mass spectra of AMPs, their fragmentation routes were rationalized. This was confirmed by the GC/MS analysis of deuterated analogues

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J Chromatogr A 2009 **1216** (20) 4319

Liquid-liquid-liquid microextraction of degradation products of nerve agents followed by liquid chromatography-tandem mass spectrometry

Important environmental markers of nerve agents are alkyl alkylphosphonic acids (AAPAs). A simple hollow fiber-based liquid-liquid-liquid microextraction (HFLLLME) method has been produced to enhance the AAPAs from water. AAPAs were extracted from acidified aqueous phase to organic phase present in pores of the hollow fiber. They were then back extracted into the alkaline acceptor phase present in the lumen of the hollow fiber. Parameters influencing the HFLLLME process were optimized using a Plackett-Burman design and a Doehlert design. Optimal extraction conditions were: organic solvent, 1-octanol; pH of acceptor phase, 14; extraction time, 60min; pH of donor phase, 1; and NaCl concentration, 10% (w/v). Depending upon the alkyl substituent, lower limits of detection varied from 0.1 to 100ng/ml (S/N>/=5). Repeatability of the technique was noted with relative standard deviation of 1.49-9.83% (n = 3). Following validation, the technique was employed to detect AAPAs present in the water sample provided by the Organization for Prohibition of Chemical Weapons (OPCW) during the 23rd Official Proficiency Test. The additional benefit of this technique is that several successive extractions of AAPAs from the same water sample can be performed

16 Workplace

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J Am Soc Mass Spectrom 2009 **20** (5) 829

Direct atmospheric pressure chemical ionization-tandem mass spectrometry for the continuous real-time trace analysis of benzene, toluene, ethylbenzene, and xylenes in ambient air

Real-time monitoring of benzene, toluene, ethylbenzene, and xylenes (BTEX) in ambient air is necessary for the early warning detection accompanying the release of these hazardous chemicals and in estimating the potential exposure risks to humans and the environment. A tandem mass spectrometry (MS/MS) method has been developed for continuous real-time determination of ambient trace levels of BTEX. The method employs sampling of air *via* an atmospheric pressure inlet directly into the atmospheric pressure chemical ionization (APCI) source. The method is linear over four orders of magnitude with correlation coefficients greater than 0.996. Low limits of detection in the range 1-2 µg/m^3 are achieved for BTEX. The reliability of the method was endorsed by the estimation of quality parameters such as repeatability and reproducibility

(relative standard deviation below 8% and 10%, respectively) and accuracy (over 95%). The applicability of this method to real-world samples was determined by measurements of BTEX levels in real ambient air samples and results compared with a reference GC-FID method. This direct APCI-MS/MS technique is suitable for real-time determination of BTEX in ambient air during regulation surveys as well as for the monitoring of industrial processes or emergency situations

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Talanta 2009 **78** (4-5) 1286
Comparative evaluation of liquid chromatography *versus* gas chromatography using a -cyclodextrin stationary phase for the determination of BTEX in occupational environments
Analysis of benzene, toluene, ethylbenzene and *o*-xylene, *m*-xylene and *p*-xylene in occupational environments was performed by HPLC and the results compared with GC-MS. HPLC employed a β-cyclodextrin stationary phase and was conducted after active and passive air sampling by adsorption on activated charcoal and pressurized fluid extraction. Whereas GC-MS provided better resolutions and lower detection limits than HPLC, the compounds were completely separated and quantified with both procedures. However, HPLC was unsuccessfully employed in the determination of benzene in real samples because its sensitivity was too low. Both techniques were employed in the analysis of certified reference materials and air samples collected in several workplace environments. Statistical comparison of HPLC and GC-MS results indicated equivalent accuracy

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Biomarkers 2009 **14** (3) 137
Analysis of dialkyl phosphate metabolites in hair using gas chromatography-mass spectrometry: A biomarker of chronic exposure to organophosphate pesticides
Three dialkyl phosphate (DAP) metabolites, dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP) and diethyl phosphate (DEP) of organophosphate pesticides (OPs) were investigated in hair samples. The procedure includes a decontamination step, solid-liquid extraction, followed by liquid-liquid extraction, pentafluorobenzyl bromide derivatization, clean-up on Florisil/PSA column and analysis by gas chromatography-mass spectrometry (GC-MS). Extraction recovery, produced with 50 mg hair samples elaborated at two concentration levels, ranged from 56.1 to 107.9% and the within-day precision ranged from 13.5 to 17.5%. Limits of detection (LODs) ranged from 0.02 to 0.10 ng/mg. Analysis of hair samples of 30 agricultural workers indicated the viability of the procedure for monitoring people occupationally exposed to OPs. DEP was the most frequently detected compound followed by DMP. This is the first report on the detection of dialkyl phosphates in human hair and demonstrates the suitability of hair testing to assess chronic OPs exposure

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J Anal Toxicol 2009 **33** (4) 223
Determination of 2,5-hexanedione in urine by headspace solid-phase microextraction and gas chromatography
The most important metabolite of *n*-hexane and methyl ethyl ketone in human urine is 2,5-hexanedione (2,5-HD). It is employed as a biomarker for biological monitoring of occupational exposure to *n*-hexane. Investigations were conducted following development of a simple method using headspace solid-phase microextraction (HS-SPME) and gas chromatography (GC) equipped with a flame-ionization detector (FID). Parameters which affect HS-SPME-GC-FID were optimized (i.e., fiber coating, sample volume, adsorption and heating time, salt addition, and extraction temperature). The assay proved linear in the range of 0.075 to 20.0 mg/l, precision (coefficient of variation < 7.0%), and detection limit of 0.025 mg/l for 2,5-HD in urine. Urine samples from eight workers occupationally exposed to *n*-hexane in shoemaker's glue were successfully analysed for 2,5-HD

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J Chromatogr A 2009 **1216** (26) 5069
Simultaneous determination of 18 pyrethroids in indoor air by gas chromatography/mass spectrometry
The simultaneous measurement of 18 pyrethroids (allethrin, bifenthrin, cyfluthrin, cypermethrin, cyphenothrin, deltamethrin, empenthrin, fenpropathrin, furamethrin, imiprothrin, metofluthrin, permethrin, phenothrin, prallethrin, profluthrin, resmethrin, tetramethrin and transfluthrin) in indoor air has been achieved by development of an analytical procedure. Pyrethroids were collected for 24 h using a combination of adsorbents (quartz fiber filter disk and Empore C_{18} disk), with protection from light, and then extracted with acetone, concentrated, and analyzed by GC/MS. Determination was accurate and precise (detection limits: ca. 1 ng/m^3). Collected pyrethroid samples may be stored for up to one month at 4°C in a refrigerator

17 Product Authenticity

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Food Addit Contam 2009 **26** (5) 595
Determination of synthetic drugs used to adulterate botanical dietary supplements using QTRAP LC-MS/MS
The use of synthetic drugs to adulterate botanical dietary supplements has become a serious problem. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) coupled with a linearity ion-trap system in the multiple reaction monitoring (MRM) plus enhanced product ion (EPI) mode has beeen developed to test for synthetic adulterants. The procedure was employed to test for 23 drugs with various pharmacological effects, comprising blood pressure and lipid-lowering agents, sedative drugs, anti-diabetic drugs, weight-reducing agents and aphrodisiac compounds. A single transition was monitored using protonated molecules as precursor ions. EPI spectra were stored in a library and recognized by library searching. Several undeclared drugs were identified in herbal remedies, e.g., glibenclamide, sibutramine hydrochloride and sildenafil. Out of a total of 105 botanical dietary supplements examined, 35 tested positive. The technique was selective, sensitive, rapid, high-throughput and reliable

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Anal Chem 2009 **81** (12) 4803
Combining two-dimensional diffusion-ordered nuclear magnetic resonance spectroscopy, imaging desorption electrospray ionization mass spectrometry, and direct analysis in real-time mass spectrometry for the integral investigation of counterfeit pharmaceuticals
Recently, counterfeit medicines have been increasingly involved in terms of the number of reported cases in developing and developed countries. Artesunate-based antimalarial drugs have been especially targeted because of their high demand and cost. Counterfeit antimalarials may result in fatalities and also contribute to the growing problem of drug resistance, particularly in southeast Asia. Analysis by means of two-dimensional diffusion-ordered ^{1}H nuclear magnetic resonance spectroscopy (2D DOSY ^{1}H NMR) with direct analysis in real-time mass spectrometry (DART MS) and desorption electrospray ionization mass spectrometry (DESI MS) was evaluated. The procedure was applied to 14 different artesunate tablets, representative of what can be purchased from informal sources in southeast Asia. However, artesunate-based antimalarial drugs were detected in only five formulations *via* both nuclear magnetic resonance (NMR) and mass spectrometry (MS) methods. Common organic excipients such as sucrose, lactose, stearate, dextrin, and starch were also detected. The graphical representation of DOSY ^{1}H NMR data facilitates the establishment of similarities within groups of samples, enabling counterfeit drug "chemotyping". In addition to bulk- and surface-average analyses, spatially resolved information on the surface composition of counterfeit and genuine antimalarial formulations was produced by DESI MS performed in the imaging mode. This enabled the visualization of the homogeneity of both genuine and counterfeit drug samples. This research indicates that 2D DOSY ^{1}H NMR when combined with ambient MS, results in a powerful suite of instrumental analysis techniques for the integral characterization of counterfeit antimalarials

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J AOAC Int 2009 **92** (3) 830
Automated chemiluminometric screening of counterfeit drugs of the antituberculosis agent pyrazinamide
A growing challenge for the authorities is presented by counterfeit drugs to prevent them from entering health systems and causing serious consequences for consumers, drug manufacturers, and governments. A simple, low-cost, and expeditious chemiluminometric approach, relying on a fully automated multipumping flow system for screening pharmaceutical preparations of the

antituberculosis drug pyrazinamide is described and implemented. The chemiluminescent method was developed and based upon the scavenging effect of pyrazinamide on the oxidation of luminol by hydrogen peroxide in alkaline medium. To monitor the analytical signal, a homemade chemiluminescence detector relying on a photomultiplier module was produced. Linear calibration plots for pyrazinamide concentrations between 10 and 70 mg/l were obtained (r = 0.9931) with good precision (RSD < 0.99%; n = 21). The detection limit was 5.79 mg/l, and the sampling rate was approximately 150 determinations per hour

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Detection of counterfeit antiviral drug Heptodin and classification of counterfeits using isotope amount ratio measurements by multicollector inductively coupled plasma mass spectrometry (MC-ICPMS) and isotope ratio mass spectrometry (IRMS)

Isotope ratio mass spectrometry (IRMS) and multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) are very important techniques which produce forensic data that might otherwise not be available. MC-ICP-MS has been demonstrated to be a very decisive method for measuring high precision and accuracy isotope amount ratios. The potential of combining isotope amount ratio measurements performed by MC-ICP-MS and IRMS has been investigated for the detection of counterfeit pharmaceuticals. The antiviral drug, Heptodin, has undergone an extensive study which been carried out for several isotopic ratios by combining MC-ICP-MS and an elemental analyser EA-IRMS for stable isotope amount ratio measurements. One hundred and thirty-nine batches of the antiviral drug were analysed for their C, S, N and Mg isotope ratios. Authenticity ranges have been produced for each isotopic system and combined to produce a unique multi-isotopic pattern only present in the genuine tablets. Those tablets with an isotopic fingerprint outside the genuine range were identified as counterfeit. Therefore, the combination of those two methods has great potential to detect counterfeit pharmaceuticals. A far greater power of discrimination is produced when at least three isotopic systems are combined. Data from these studies could be employed as evidence in court. Therefore, the methods require validation to support their reliability. In addition, it is vital to be able to produce uncertainty values associated with the isotope amount ratio measurements so that significant differences may be identified and the authenticity of a sample may be assessed

18 Techniques

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HPTLC analysis of a new ultra-short-acting thiazolodiazepine hypnotic (HIE-124) in spiked human plasma

Amongst the new generation of ultra-short-acting hypnotics is ethyl 8-oxo-5,6,7,8-tetrahydrothiazolo[3,2-*a*][1,3]diazepin-3-carboxylate (HIE-124). The first quantitative analysis of HIE-124 in plasma is reported following the development of a high-performance thin-layer chromatographic (HPTLC) technique. Baseline separation between HIE-124 and diazepam (internal standard) was accomplished on HPTLC plates with chloroform-ethyl acetate 80:20 (*v/v*) as mobile phase. Densitometric detection was performed at 265 nm. The method was linear in the range 40-400 µg/ml (r = 0.997) and recoveries of the drug were in the range 93-102% at 80-350 µg/ml. Within-run and between-run precision estimated as RSD [%], and accuracy calculated as percentage error were in the ranges 2.1-4.6% and 0.8-6.9%, respectively. The limits of detection (LOD) and quantification (LOQ) in spiked human plasma were 20 and 40 µg/ml (40 and 80 ng per band), respectively

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Comparison of drugs of abuse detection in meconium by EMIT II and ELISA

A comparison has been made between the results of meconium specimens and fortified samples screened for drugs of abuse by both enzyme multiplied immunoassay technique (EMIT® II) and enzyme-linked immunosorbent assay (ELISA) methods. Sample preparation for the ELISA screen was a simple buffer extraction whereas the EMIT II screen was a lengthy and more laborious sample preparation procedure. The ELISA method was automated using a TECAN Genesis and the EMIT II analysis was automated with an Olympus AU400e. Calibration involved hydromorphone for the opioid screen and clonazepam for the benzodiazepine screen to maximize detection for these analytes. Previously validated gas chromatography-mass spectrometry (GC-MS), two-dimensional GC-MS, or liquid chromatography-tandem MS methods were used for confirmation. Results from the two techniques compared well. Agreement of the ELISA assay was greater than 90% when compared with EMIT II for all drug classes except barbiturates and benzodiazepines. ELISA appears to be more sensitive than EMIT II for the detection of amphetamines, methadone, propoxyphene, and cocaine. ELISA compared favorably with EMIT II for cannabinoids, opioids, and PCP. Specificity of the ELISA assay was slightly greater for PCP and opioids. EMIT II appears to be more sensitive for the detection of barbiturates and benzodiazepines. The ELISA method reduced turnaround time by 50% compared to EMIT II

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The limit of detection in generalized least-squares calibrations: An example using alprazolam liquid chromatography-tandem mass spectrometry data

When analytes are at low levels, limit of detection (LOD) values provide useful indicators for the applicability of an analytical technique. In addition, a LOD value may be employed to estimate the false positive probability x($p_{x\ >or=\ LOD}$) of a result for a sample where no analyte is present, as well as the false negative probability ($p_{x\ <or=\ 0}$) for a sample analyte level at the LOD. For straight line least-squares calibrations, the LOD of a calculated concentration (LOD_x) results from uncertainties in low-level signal (y) measurements and in the calibration intercept (a_1) and slope (a_2) parameters. Using generalized least-squares (GLS) calibrations, uncertainties in both the concentration (x_i) and signal (y_i) calibration data contribute to the fit parameter uncertainties. LOD_x was employed as 3 $\sigma_{x\ =\ 0}$, where the calculated standard deviation, $\sigma_{x\ =\ 0}$, incorporates all the uncertainty contributions. The GLS data may be interpreted in terms of small nonlinear distortions of the weighted ordinary least-squares (WOLS) problem. The distortions result in skewed distributions of calculated x values and so exact GLS results for the asymmetric values were obtained. Differences between WOLS and GLS approaches are least when calibration uncertainties in x_i are small, as with alprazolam here. A second example, employing synthetic data from earlier GLS work, demonstrates greater differences between $\sigma_x^{+/-}{}_{\ =\ 0}$ and σ_x. Monte Carlo calculations for this example show that GLS-derived false positive and negative probabilities may differ by factors of two or more from normal distribution predictions, even when $\sigma_x^{+}{}_{\ =\ 0}$ is employed to replace $\sigma_{x\ =\ 0}$ in the LOD definition. For the user, spreadsheet or other computational implementations of the GLS approach are as easy to employ compared with those for a WOLS treatment. The GLS results will be the same as WOLS results when x uncertainties are excluded. Consequently, since GLS is a more complete and warranted method, it should be employed as the standard procedure for weighted least-squares calibrations

Schramm E, Kurten A, Holzer J, Mitschke S, Muhlberger F, Sklorz M, Wieser J, Ulrich A, Putz M, Schulte-Ladbeck R, Schultze R, Curtius J, Borrmann S, Zimmermann R*// *Helmholtz Zentrum Munchen, Inst Ecol Chem, Ingolstadter Landstr 1, DE-85764 Neuherberg, Germany

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Trace detection of organic compounds in complex sample matrixes by single photon ionization ion trap mass spectrometry: Real-time detection of security-relevant compounds and online analysis of the coffee-roasting process

An in-house-built ion trap mass spectrometer including a soft ionization source has been developed. Single-photon ionization was achieved with an ionization source of an electron beam pumped vacuum UV (VUV) excimer lamp (EBEL). Soft ionization was shown to facilitate the reduction of fragmentation of the target analytes and the suppression of most matrix components. Consequently, the combination of photon ionization with the tandem mass spectrometry (MS/MS) capability of an ion trap produces a powerful tool for molecular ion peak detection and identification of organic trace compounds in complex matrixes. The technique was successfully tested in two different situations. Firstly, the detection of security-relevant substances like explosives, narcotics, and chemical warfare agents. From within each of these groups, one test substance was selected and detected successfully with single photon ionization ion trap mass spectrometry (SPI-ITMS) MS/MS measurements. In addition, tests were first performed to demonstrate that this method was not influenced by matrix compounds. Secondly, the detection of process gases. Exhaust gas from coffee roasting was investigated in real time, and some of its compounds were identified using MS/MS studies