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Current awareness in drug testing and analysis

1 Reviews

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Ther Drug Monit 2008 **30** (2) 225
The role of liquid chromatography-tandem mass spectrometry (LC-MS/MS) to test blood and urine samples for the toxicological investigation of drug-facilitated crimes
An overview is presented of the drug-facilitated crime (DFC) phenomenon, particularly in France. Recently, there has been an increase in incident report (mainly sexual assaults and robbery), scientific publications and congress presentations. Following national enquiries, a list of drugs reportedly associated with DFC was established and includes benzodiazepines and benzodiazepine-like drugs (zolpidem, zopiclone), minor tranquilizers and neuroleptics, barbiturates, narcotics, hallucinogens, and anaesthetics. Some of these molecules are specific to France in DFC cases. Using healthy volunteers who had taken benzodiazepines (lorazepam, bromazepam, flunitrazepam, clonazepam), zolpidem and zopiclone, a study showed that the only way to increase the period of detection of these drugs was to use liquid chromatography-tandem mass spectrometry (LC-MS/MS) to test blood and urine samples. The very high sensitivity of this method appears to be an essential condition to document the cases, because the drugs tested were still detectable in urine at least 6 days after the ingestion of one therapeutic dose. Limits of detection were always lower than 0.5 ng/mL in urine. The actual list of molecules and metabolites the authors screened for in urine and blood by LC-MS/MS, in every DFC, is provided in detail: 25 benzodiazepines and benzodiazepine-like drugs, 11 minor tranquilizers and neuroleptics, 2 barbiturates, 12 narcotics, 4 hallucinogens, and 1 anaesthetic. However, the distinction between continual therapeutic use of a psychotropic drug or illegal narcotic and a single ingestion should be documented by sequential analysis of hair, again with LC-MS/MS

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Anal Bioanal Chem 2008 **391** (1) 97
LC-MS-based procedures for monitoring of toxic organophosphorus compounds and verification of pesticide and nerve agent poisoning
Organophosphorus compounds (OPCs) are used worldwide as, e.g., flame retardants, plasticizers, and pesticides and remaining stockpiles of OPC nerve agents are present in military arsenals. They exhibit acute and potential chronic toxicity to man, the environment, and biota thus emphasizing the need for efficient analytical procedures to monitor potential risk to health. This review discusses LC-MS-based techniques for OPC detection, addressing sample preparation, separation, ionization, and detection in comprehensive detail. For sample preparation conventional liquid-liquid extraction (LLE) and diverse solid-phase extraction (SPE) techniques are still regularly employed. However, during the last three years a number of sophisticated novel techniques have been developed. Solid-phase microextraction (SPME), stir-bar-sorptive extraction (SBSE), membrane-assisted solvent extraction (MASE), and specifically designed molecularly imprinted polymers (MIP) exhibit high potential for frequent use in the future. In this review, additional emphasis is dedicated to the recent history and current progress in ionization and MS detection of OPCs. The number of relevant published LC-MS reports has tripled in the last five years. This is particularly the result of the proliferationg in the use of electrospray ionization (ESI), currently an indispensable and reliable tool for LC-MS coupling. LC-MS is becoming an appropriate complementary or replacement method for the more traditional GC-MS methods, and not only for non-volatile, hydrophilic, and ionic OPCs. The last section of this review covers recent techniques for verification of OPC poisoning. LC-MS-MS detection of phosphylated peptides generated from inhibited circulating serum butyrylcholinesterase (BChE) by valuable proteomics methods enables proof of intoxication on the molecular level. Therefore, this review gives a comprehensive overview on the status quo of LC-MS-based OPC analysis in respect of both technical progress and relevant applications

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Mass Spectrom Rev 2008 **27** (1) 35
Mass spectrometric determination of insulins and their degradation products in sports drug testing
Insulins' anabolic and anti-catabolic properties have supposedly led to its misuse in sport. Hence, doping control assays were developed to allow the unequivocal identification of synthetic insulin analogs and metabolic products derived from human insulin and its artificial counterparts in urine and plasma specimens. Analyses were based on immunoaffinity purification and subsequent characterization of target analytes by top-down sequencing-based approaches, which were conducted with hybrid tandem mass spectrometers that consisted of either quadrupole-linear ion trap or linear ion trap-orbitrap analyzers. Diagnostic product ions and analytical strategies are presented and discussed in light of the need to unambiguously identify misused drugs in urine and plasma specimens for doping control

2 Sports Doping - General

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J Anal Toxicol 2008 **32** (3) 232
Analysis of confiscated black market drugs using chromatographic and mass spectrometric approaches

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.

Numerous drugs were confiscated during house searches in Germany and subjected to chemical analysis. They included anabolic agents such as various anabolic-androgenic steroids (stanozolol, testosterone derivatives, trenbolone esters, etc.) and clenbuterol, as well as agents with anti-estrogenic activity (tamoxifen, clomiphene), drugs stimulating virility (sildenafil, tadalafil), and unlabeled plastic bags. Liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry with nitrogen-phosphorus specific detection, gel electrophoresis, and immunological tests were utilised to test for the effective content of 70 products. In 18 cases (25.7%), the declared ingredients differed from the actual content, especially supposed anabolic-androgenic steroids. Nandrolone and trenbolone esters, for example, were frequently substituted or complemented by various testosterone derivatives, and several testosterone depot formulations originally composed of four different esters were found to contain fewer or incorrect components. With the exception of those drugs supposedly originating from so-called underground labs, fake packings were hardly or not distinguishable from original boxes following visual inspection

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Eur J Mass Spectrom 2008 **14** (3) 117
Quantitative analysis of urinary glycerol levels for doping control purposes using gas chromatography-mass spectrometry
Increased fluid retention and improved performance results from the administration of glycerol to endurance athletes especially under hot and humid conditions. Therefore, glycerol is considered relevant for sports drug testing and methods for its analysis in urine specimens is necessary. However, a major concern is the natural occurrence of trace amounts of glycerol in human urine, which requires a quantitative analysis and the determination of normal urinary glycerol levels under various sporting conditions. A quantitative method was developed using a gas chromatography/isotope-dilution mass spectrometry-based approach that was validated with regard to lower limit of detection (0.3 μg mL^{-1}), lower limit of quantification (0.9 μg mL^{-1}), specificity, linearity (1.0-98.0 μg mL^{-1}), intraday and interday precision (<20% at 2.4, 24.1 and 48.2 μg mL^{-1}) as well as accuracy (92-110%). Sample aliquots of 20 μL were spiked with five-fold deuterated glycerol, dried and derivatised using *N*-methyl-trimethylsilyltrifluoroacetamide (MSTFA) before analysis. The established method was employed to analyse a total of 1039 doping control samples covering various sport disciplines (349 endurance samples, 286 strength sport samples, 325 game sport samples and 79 other samples) in- and out-of-competition, which provided quantitative information about the glycerol content commonly observed in elite athletes' urine samples. About 85% of all specimens provided glycerol concentrations < 20.0 μg mL^{-1} and few reached values up to 132.6 μg mL^{-1}. One further sample, however, was found to contain 2690 μg mL^{-1}, which might indicate the misuse of glycerol, but no threshold for urinary glycerol concentrations has been established to date due to the lack of substantial data. On the basis of the results obtained from the studied reference population, a threshold for glycerol levels in urine set at 200 μg mL^{-1} is suggested. This provides a tool to doping control laboratories to test for the misuse of this agent in elite and amateur sport

3 Steroids

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J Chromatogr A 2008 **1190** (1-2) 278
Multivariate optimization of a derivatisation procedure for the simultaneous determination of nine anabolic steroids by gas chromatography coupled with mass spectrometry
The medical commission of the International Olympic Committee prohibits the use of anabolic androgenic steroids to improve sporting performances. Nine anabolic steroids (androsterone (A), nandrolone, estradiol, testosterone propionate, nandrolone-17 propionate, dydrogesterone, testosterone, epitestosterone, boldenone) and α-cholestane as internal standard were analysed by gas chromatography coupled with mass spectrometry (GC/MS). Derivatisation of anabolic steroids was achieved with a mixture of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), ammonium iodide and 2-mercaptoethanol (1000:2:6, v/w/v). Trimethylsilyl (TMS) derivatives resulted. Anabolic steroids can be derivatised into one or two forms, mainly for androsterone into A-monoTMS and A-diTMS. This study was intended to investigate the optimization conditions of the derivatisation process (maximum yield of silylation reaction) of each anabolic steroid into only one form. A two-level factorial Doelhert design was used to determine the influence of different parameters and their interactions on each compound by means of response surface methodology. The parameters to be optimized were the reaction time and the temperature. The interaction "temperature-reaction time" is significant and has a positive effect on the improvement of the effectiveness of the derivatisation. In respect of the large amount of information, often not convergent, a global desirability function was applied for multi-responses optimization. Thus, the optimized temperature and the reaction time of silylation were 85°C and 24 min, respectively. Several GC/MS analytical parameters were also investigated: linearity (regression coefficient upper than 0.99 for each compound, sensibility (range of concentration 0.05-0.30 μg/ml). Confirmatory experiments were employed to validate the predicted values and to confirm the model. The validation assay responses are relatively close to the responses predicted. Satisfactory resolutions by GC/MS and a run lower than 12 min were observed

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Drug Metab Dispos 2008 **36** (3) 571
Aryl-propionamide-derived selective androgen receptor modulators: Liquid chromatography-tandem mass spectrometry characterization of the *in vitro* synthesized metabolites for doping control purposes
Selective androgen receptor modulators (SARM) are a notable group of compounds misused in sports because of their beneficial anabolic properties and reduced side effects. In preventive doping control analysis of relevant compounds, the challenge is to predict their metabolic fate. For aryl-propionamide-derived SARM, an *in vitro* assay using microsomal and S9 human liver enzymes was produced to simulate phase-I and phase-II metabolic reactions. *in vitro* metabolic profiles and the structure-metabolic relationship were compared between four structurally modified substrates. Accurate mass measurements were employed to characterize the synthesized metabolites. In addition, collision-induced dissociation was employed to indicate a methodological approach to monitor the prohibited use of aryl-propionamide-derived drug candidates. Further phase-I and phase-II metabolic reactions were successfully combined in one *in vitro* assay. The main pathway of phase-I modifications utilized the hydrolysis of the ether linkage, monohydroxylation, and hydrolytic cleavage of the amide bond. Nitro-reduction and deacetylation were reactions noted for substrates possessing the corresponding functionality. SARM metabolites were analyzed in negative ion electrospray ionization and detected as deprotonated species $[M-H]^-$. Metabolic modifications were primarily observed to involve the B-ring side, and collision-induced dissociation induced product ions originating from the A-ring side of the compound. These structure-specific ions may be employed as target ions in the routine doping control

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Eur J Mass Spectrom 2008 **14** (3) 181
Screening of *in vitro* synthesised metabolites of 4,9,11-trien-3-one steroids by liquid chromatography-mass spectrometry
In order to study and predict the metabolic patterns of new derivatives of anabolic androgenic steroids (AAS), a flexible *in vitro* synthesis procedure was developed to analyse most prominent target compounds for doping control purposes. Microsomal and S9 fraction of human liver preparations were employed as a source of metabolising enzymes and the co-substrates of the synthesis mixture were selected to favour phase-I metabolic reactions and glucuronidation as phase-II conjugation reactions. Model compounds within the study were 4,9,11-trien-3-one steroids, structural derivatives of gestrinone and trenbolone, both of which are included in the list of prohibited compounds in sports by the World Anti-Doping Agency (WADA). The correlation between *in vitro* metabolism of human microsomes and *in vivo* excretion studies in human was compared with gestrinone. Subsequently, the applicability of the *in vitro* model for prediction of AAS metabolic pathways for new doping agents was evaluated. All the AAS examined within this study were successfully metabolised using the developed *in vitro* model, hydroxylation, reduction and glucuronide conjugation being the most prominent reaction pathways. Hydroxylated and glucuronide-conjugated metabolites of *in vivo* experiment with gestrinone were the same metabolites formed in the enzyme-driven process, thereby showing good *in vitro-in vivo* correlation. Liquid chromatographic-mass spectrometric and tandem mass spectrometric methods were developed, relying on the positive polarity of electrospray ionisation. This also allowed the direct detection of intact glucuronide-conjugated AAS metabolites. Due to charge delocalisation and high proton affinity, the described method was proven effective in the analysis of AAS metabolites bearing extensive conjugated double bond systems in their structures

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Eur J Mass Spectrom 2008 **14** (3) 171
A mass spectrometric approach for the study of the metabolism of clomiphene, tamoxifen and toremifene by liquid chromatography time-of-flight spectroscopy
The capabilities of liquid chromatography coupled to mass spectrometry with a time-of flight system with accurate mass measurement for the detection and characterisation of drug metabolites in biological samples for anti-doping purpose is described. Urinary excretion samples of three selective oestrogen receptor modulators (SERMs) with a common triphenylethylene structure: clomiphene, toremifene, and tamoxifen, obtained after oral administration of a single dose of each drug, were analysed using a time-of-flight system, after automatic tuning and calibration of the equipment, in positive full scan mode

using an electrospray ionisation source. Most significant metabolites reported by others and postulated new metabolites were identified, especially those of toremifene, was characterised: *N*-demethyl-3-hydroxy-4-methoxy-toremifene and 3- hydroxy-4-methoxy-toremifene. Additionally, in the urinary excretion samples of toremifene, some metabolites without the characteristic chlorine isotope pattern and discarded in previous studies were identified. The lack of certified reference materials does not allow an accurate determination of the limit of detection (LODs) of all metabolites. However, an estimation taking into account the response factor of similar compounds enabled an estimate that all metabolites are clearly detectable in a range of concentration comprised between 10 ng mL^{-1} and 30 ng mL^{-1}

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Rapid Commun Mass Spectrom 2008 **22** (3) 321
6 -Methylandrostenedione: Gas chromatographic mass spectrometric detection in doping control
In recent years products containing 6α-methylandrost-4-ene-3,17-dione have appeared on the sport supplement market. Scientific studies have proven aromatase inhibition and anabolic and mild androgenic properties; however, no preparation has been approved for medical use up to now. In sports 6α-methylandrost-4-ene-3,17-dione has to be classified as a prohibited substance according to the regulations of the World Anti-Doping Agency (WADA). For the detection of its misuse the metabolism was studied following the administration of two preparations obtained from the Internet (Formadrol and Methyl-1-Pro). Several metabolites as well as the parent compounds were synthesized and the structures of 3α-hydroxy-6α-methyl-5β-androstan-17-one, 6α-methylandrost-4-ene-3,17-dione, and 5β-dihydromedroxyprogesterone were confirmed by nuclear magnetic resonance (NMR) spectroscopy. The main metabolite, 3α-hydroxy-6α-methyl-5β-androstan-17-one, was found to be excreted as glucuronide and was still detectable in µg/mL amounts until urine collection was terminated (after 25 h). Additionally, samples from routine human sports doping control had already tested positive for the presence of metabolites of 6α-methylandrost-4-ene-3,17-dione. Screening analysis can be easily performed by the existing screening procedure for anabolic steroids using 3α-hydroxy-6α-methyl-5β-androstan-17-one as target substance (limit of detection < 10 ng/mL). Its discrimination from the closely eluting drostanolone metabolite, 3α-hydroxy-2α-methyl-5α-androstan-17-one, is possible as the mono-TMS derivative

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Anal Chim Acta 2008 **613** (2) 228
Profiling of 19-norsteroid sulfoconjugates in human urine by liquid chromatography mass spectrometry
Sulfoconjugated and glucuroconjugated forms of 19-nortestosterone (nandrolone) are the major metabolites excreted in human urine. A sensitive and selective liquid chromatography/tandem mass spectrometry (LC/MS/MS) technique in negative ESI mode was developed for direct quantification of 19-norandrosterone sulfate (19-NAS) and 19-noretiocholanolone sulfate (19-NES). For both sulfoconjugates, the $[M-H]^-$ ion at *m/z* 355 and the fragment ion at *m/z* 97 were used as the precursor and product ions, respectively. The isolation technique employed a complete and rapid separation of sulfates and glucuronides in two extracts after loading the sample on a weak anion exchange solid phase extraction support (SPE Oasis WAX). Thereafter, sulfates were separated by LC (Uptisphere ODB, 150 mm x 3.0 mm, 5 µm) and analyzed on a linear trap and a triple quadrupole mass spectrometer. The lower limit of detection (LLOD) and lowest limit of quantification (LLOQ) were of 100 pg mL^{-1} and 1 ng mL^{-1}, respectively. Assay validation demonstrated good performances in terms of trueness (92.0-104.9%), repeatability (0.6-7.2%) and intermediate precision (1.3-10.8%) over the range of 1-2500 ng mL^{-1}. Finally, after intake of 19-norandrostenedione (nandrolone precursor), 19-NAS and 19-NES in urine samples collected were quantified. This assay may be easily employeded to separate glucuronide and sulfate steroids from urine specimens prior to quantification by LC/MS/MS

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Anal Bioanal Chem 2008 **391** (1) 251
Determination of benzimidazole- and bicyclic hydantoin-derived selective androgen receptor antagonists and agonists in human urine using LC-MS/MS
Selective androgen receptor modulators (SARMs) represent a novel class of drugs with tissue-specific agonistic and antagonistic properties. They are prohibited in sports from January 2008 according to the World Anti-Doping Agency. Preventive approaches to reduce the use of SARMs include early implementation of target analytes into doping control screening assays. Five model SARMs were synthesized, four of which are analogs to prostate-specific androgen receptor antagonists with a 5,6-dichloro-benzimidazole nucleus. The fifth SARM is a muscle-tissue specific agonist with a bicyclic hydantoin structure (BMS-564929). Dissociation pathways after negative electrospray ionization were studied using an LTQ-Orbitrap mass analyzer, and diagnostic product ions and common fragmentation patterns were utilised to establish a screening procedure that target the intact SARMs as well as supposed metabolic products. Sample preparation employing solid-phase extraction and subsequent LC-MS/MS measurement allowed for detection limits of 1-20 ng/mL, intra- and interday precisions of between 2.4 and 13.2% and between 6.5 and 24.2%, respectively. Recoveries varied from 89 to 106%, and tests for ion suppression or enhancement effects were negative for all analytes

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Eur J Mass Spectrom 2008 **14** (3) 153
Screening for two selective androgen receptor modulators using gas chromatography-mass spectrometry in doping control analysis
Selective androgen receptor modulators (SARMs) resulting in the tissue-selective stimulation of androgen receptorshave become a major field of clinical research. Promising results have been obtained from clinical trials during the treatment of debilitating diseases, osteoporosis and frailty. However, the potential for misuse of SARMs in sport is notable and drug testing methods based on liquid chromatography were established for different classes including arylpropionamide-, 2-quinolinone- and bicyclic hydantoin-derived compounds. As gas chromatography and mass spectrometry (GC-MS) are still important analytical techniques in sports drug testing, a method to determine 2-quinolinone- and bicyclic hydantoin-derived SARMs established. Spiked urine samples were analysed by routine doping control protocols including enzymatic hydrolysis, liquid-liquid extraction, concentration and derivatisation to trimethylsilylated analogues followed by GC-MS analysis. The technique was validated for the items specificity, lower limit of detection (0.2-10 ng mL^{-1}), recovery (83-85%), intraday and interday precision (9-15% and 13-18%, respectively), which illustrates the suitability of conventional GC-MS systems to determine representatives of an emerging class of compounds in doping control specimens

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Eur J Mass Spectrom 2008 **14** (3) 163
Use of an electrochemically synthesised metabolite of a selective androgen receptor modulator for mass spectrometry-based sports drug testing
The pathways of metabolism of new therapeutics is a major task for pharmaceutical companies and of great interest for drug testing laboratories. The latter necessitates demonstation of the presence or absence of drugs or their metabolic products in urine to test for a misuse of these compounds. Frequently, *in vitro* or animal models are used to mimic the human metabolism and produce potential targets in amounts allowing for method development. Alternatively, electrochemical reactions of drugs was reported to allow for the generation of selected metabolites. The utility of this approach for doping control purposes was illustarted with a novel class of anabolic agents termed selective androgen receptor modulators (SARMs). An arylpropionamide- derived drug candidate was subjected to electrochemical "metabolism" and a major phase-I- metabolite, resulting from the elimination of a substituted phenol residue as identified in *in vitro* experiments, was generated and characterised using liquid chromatography/nuclear magnetic resonance spectroscopy and high resolution/high accuracy mass spectrometry. The metabolite has been included in routine doping control procedures based on liquid chromatography/tandem mass spectrometry and has provided a reference compound for 5000 doping control specimens

4 Peptides

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Rapid Commun Mass Spectrom 2008 **22** (4) 477
Quantification of human insulin-like growth factor-1 and qualitative detection of its analogues in plasma using liquid chromatography/electrospray ionisation tandem mass spectrometry
Human insulin-like growth factor-1 (IGF-1) is a peptide hormone that acts as a mediator of most of the somatotropic effects of growth hormone (GH). Therefore, it is supposed to be a biomarker indicating GH abuse in sports as well as diseases associated with a change in IGF-1 plasma concentration. It can be applied locally by injection to increase total protein and DNA content in tissues such as skeletal muscle - a highly desirable effect in various sports disciplines. In order to improve its growth-promoting properties, the primary structure of IGF-1 has been modified, yielding analogues such as des(1-3)IGF-1 and LONG™R^3IGF-1, which show a considerably reduced affinity to the respective binding proteins in plasma and, thus, an increased bioavailability at target tissues. Due to their capability to enhance performance, IGF-1 and its analogues belong to the prohibited list of the World Anti-Doping Agency. Hence, it was necessary to develop a reliable assay for the quantification of human IGF-1 as well as the detection of its derivatives. Immunoaffinity isolation and purification from 60 µL of plasma followed by

liquid chromatography/electrospray ionisation tandem mass spectrometry enabled the unequivocal determination of all target analytes. Diagnostic product ions were characterised utilising an Orbitrap mass spectrometer with high resolution/high accuracy properties and employed for triple quadrupole MS/MS analysis. The described assay provided lower limits of detection (LLODs) between 20 and 50 ng/mL, recovery rates between 34-43% and a precision <15% at the LLOD as well as higher concentration levels. In order to prove the applicability of the developed assay, human plasma samples were analysed and the results were compared with the values obtained from a commercially available immunoradiometric assay (IRMA). Four of six samples resulted in concentration ratios with good correlation between both assays, whereas the absolute concentrations were lower for the presented procedure

5 Diuretics

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Eur J Mass Spectrom 2008 **14** (3) 127
Identification and quantification of the osmodiuretic mannitol in urine for sports drug testing using gas chromatography-mass spectrometry
Owing to its urine diluting effect and the possibility to decrease bodyweight, the osmodiuretic mannitol can be potentially misused in sports. To confirm a doping offence, resulting urinary mannitol concentrations after a prohibited intravenous application and a permitted oral intake have to be differentiated. Therefore, a reliable gas chromatography-mass spectrometry (GC-MS) method was developed based on peracetyl derivatives of the analytes. All possible hexitols (allitol, galactitol, iditol, altritols, sorbitol and mannitol) that may occur in human urine were separated and anlysed on a phenylmethylpolysiloxane column (HP-5MS) within 10.75 min, and the method illustrated its capability for quantification purposes. The lower limit of detection and lower limit of quantification were estimated at 0.9 µg mL^{-1} and 2.4 µg mL^{-1}, respectively, and the assay was confirmed for mannitol and sorbitol regarding the parameters specificity, linearity, intra- (<10%) and inter-day precision (<15%) and accuracy (92-102%). To analuse urinary mannitol concentrations after oral intake, the technique was applied to an excretion study, providing a mean urinary excretion of mannitol of 19.5%. Comparison of theoretically expected urinary levels after a common therapeutic dose of mannitol and preliminary results on physiological urinary mannitol levels were promising in respect of a threshold level for mannitol that can be utilised for doping control purposes

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Talanta 2008 **75** (3) 658
Liquid-phase microextraction combined with liquid chromatography-electrospray tandem mass spectrometry for detecting diuretics in urine
Hollow fiber liquid-phase microextraction (LPME) combined with liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) was employed to detect trace amounts of diuretics in human urine. Chromatography was performed on a C_8 reversed-phase column. A 25 µL *n*-octanol was emplyed to extract analytes in urine. Extraction was optimized using a pH 2 solution spiked with 0.15 g/mL NaCl for 40 min at 40ºC with 1010 rpm stirring. The limits of detection of diuretics in urine were 0.3-6.8 ng/mL, and linearity range was 1-1000 ng/mL. Recoveries of spiked 50 ng/mL diuretics were 97.7-102.5%. The intra-day precision and inter-day precision were 3-18% and 4-21%, respectively. The diuretics concentration profiles in patient urine were also determined. The results of this study illustrate the adequacy of LPME-LC-MS/MS method for analyzing diuretics in urine and quantification limits exceed World Anti-Doping Agency requirements

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Eur J Mass Spectrom 2008 **14** (3) 191
High-throughput and sensitive screening by ultra-performance liquid chromatography tandem mass spectrometry of diuretics and other agents
The reliability of ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) for high throughput screening in anti-doping control has been tested. A technique to screen for the presence of diuretics and other doping agents in urine has been optimised and validated. The extraction procedure employed alkaline extraction (pH 9.5) with ethyl acetate and salting-out effect (sodium chloride). The extracts were analysed by UPLC-MS/MS. Analysis of 34 forbidden drugs and metabolites was achieved in a total run time of 5 min, using a C_{18} column (100 mm x 2.1 mm i.d., 1.7 µm particle size) and a mobile phase containing deionised water and acetonitrile with formic acid, with gradient elution at a flow-rate of 0.6 mL min^{-1}. The compounds were identified by multiple reaction monitoring, using electrospray ionisation in positive- or negative-ion mode. Precursor and product ions were studied for each compound and cone voltage and collision energy were optimised. As a result of the different chemical structure of the compounds under study, extraction recoveries varied from less than 10% to 100% depending on the analyte. The limits of detection ranged from 50 ng mL^{-1} to 200 ng mL^{-1}, and all the compounds complied with the requirements of quality established by the World Anti-doping Agency. Intra-assay precision was evaluated at two concentrations for each compound and, in most cases, a relative standard deviation of the signal ratio lower than 20% resulted. The method has proved to be reliable when analysing routine samples and the short analysis time resulting from a simple sample preparation and a rapid instrumental analysis enable a fast turn-around time which makes it of great interest for routine anti-doping control purposes

6 CNS Agents

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Eur J Mass Spectrom 2008 **14** (3) 145
Doping control analysis of methoxyphenamine using liquid chromatography-tandem mass spectrometry
The regulations of the World Anti-Doping Agency (WADA) forbids the use of methoxyphenamine (*o*-methoxy-*N*,α-dimethylphenethylamine, Orthoxine), a compound used in earlier times as a bronchodilator. The drug and several of its metabolites are commonly analysed in doping control screening assays by means of gas chromatography-mass spectrometry following extraction from urine specimens. A complementary method employing liquid chromatography-atmospheric pressure chemical ionisation-tandem mass spectrometry and direct injection of urine aliquots has been developed which provided a fast and sensitive alternative to confirm the presence of the prohibited compound and degradation products in sports drug testing samples. The chromatographic separation of the active drug from isomeric compounds such as the designer drug *p*-methoxymetamphetamine (PMMA) was of particular interest to unambiguously identify the applied substance and was accomplished using a C6-phenyl reverse-phase column with isocratic elution. The established procedure was confirmed for methoxyphenamine in respect of specificity, limit of detection (0.7 ng mL^{-1}), intraday- and interday precision (2.5-5.8% and 10.8-16.2%, respectively) and its applicability was illustrated with an authentic doping control sample which tested positive for the prohibited compound early in 2008

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Eur J Mass Spectrom 2008 **14** (3) 135
Determination of selected stimulants in urine for sports drug analysis by solid phase extraction *via* cation exchange and means of liquid chromatography-tandem mass spectrometry
Stimulatory substances misuse in elite sport has been frequently reported during the last few decades and have the potential as performance enhancing agents. An analytical method was developed for the qualitative determination of selected stimulants containing a primary or secondary amine moiety in human urine for doping control purposes. A rapid and highly specific procedure based on a sample preparation using weak cation exchange solid phase extraction (SPE-XCW) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a C6-Phenyl analytical column allowed the unambiguous identification of the target analytes down to low ng mL^{-1} concentration levels. Confirmation provided recovery rates of better than 75%, precisions of less than 20% and a linear approximation in the required working range (10-750 ng mL^{-1}) were obtained for 19 different target compounds. This technique provides a rugged and highly specific alternative to the established method utilising gas or liquid chromatography after liquid-liquid extraction

7 Equine

Colas C, Garcia P, Popot MA*, Bonnaire Y, Bouchonnet S// *LCH, 15 rue Paradis, FR-91370 Verrieres le Buisson, France
J Chromatogr Sci 2008 **46** (2) 174
Optimization of solid-phase extraction for the liquid chromatography-mass spectrometry analysis of harpagoside, 8-*para*-coumaroyl harpagide, and harpagide in equine plasma and urine
Solid-phase extraction cartridges routinely used for screening in horse doping analyses were tested to optimize the isolation of harpagoside (HS), harpagide (HG), and 8-*para*-coumaroyl harpagide (8PCHG) from plasma and urine. Extracts are analyzed by liquid chromatography coupled with multi-step tandem mass spectrometry. The isolation process for plasma employed BondElut PPL cartridges and provided recoveries between 91% and 93%, with RSD values between 8 and 13% at 0.5 ng/mL. Two different procedures are required to extract analytes from urine. HS and 8PCHG were extracted using AbsElut Nexus cartridges, with recoveries of 85% and 77%, respectively (RSD between 7% and 19%). The extraction of HG employed the use of two cartridges: BondElut PPL and BondElut C18 HF, with recovery of 75% and RSD between 14% and 19%. The applicability of the extraction methods was determined on genuine equine plasma and urine samples after harpagophytum or harpagoside administration

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J Chromatogr A 2008 **1201** (2) 183
Doping control analysis of insulin and its analogues in equine plasma by liquid chromatography-tandem mass spectrometry
Prior to sports events or during the recovery phases, insulin administration may increase muscle glycogen by utilising hyperinsulinaemic clamps and increase muscle size by its chalonic action to inhibit protein breakdown. A method to detect effectively the use of insulins in horses is required in order to control insulin abuse in equine sports. in addition, the readily available human insulin and its synthetic analogues, structurally similar insulins from other species may also be used as doping agents. This investigation describes a technique for the simultaneous detection of bovine, porcine and human insulins, as well as the synthetic analogues Humalog (Lilly) and Novolog (Novo Nordisk) in equine plasma. Insulins were isolated from equine plasma by immunoaffinity purification, followed by centrifugal filtration, and analysed by nano-liquid chromatography-tandem mass spectrometry (LC/MS/MS). Insulin and analogues were detected and confirmed by comparing their retention times and major product ions. All five non-equine insulins (human insulin, Humalog, Novolog, bovine insulin and porcine insulin) were detected and confirmed at 0.05ng/mL. This technique was successful in confirming the presence of human insulin in plasma collected from horses up to 4h after having been administered a single low dose of recombinant human insulin (Humulin R, Eli Lilly). To our knowledge, this is the first identification of exogenous insulin from post-administration horse plasma samples

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Rapid Commun Mass Spectrom 2008 **22** (3) 355
Insulins in equine urine: Qualitative analysis by immunoaffinity purification and liquid chromatography/tandem mass spectrometry for doping control purposes in horse-racing
Insulin is a peptide hormone consisting of two peptide chains (A- and B-chain) that are cross-linked by two disulfide bonds. To obtain improved pharmacokinetic onset of action profiles of insulin treatment in diabetic patients, recombinant long-, intermediate-, and rapid-acting insulin analogs are produced, in which the C-terminal end of the B-chain plays an especially important role.A review of the veterinary literature reveals the low prevalence of equine type I diabetes mellitus, which indicates that the therapeutic use of insulin in racing horses is unlikely. Although there is no unequivocal evidence of an overall performance-enhancing effect of insulin, in human sports the misuse of insulin preparations is reported among elite athletes. The desired effects of insulin include the increase of muscular glycogen prior to sports event or during the recovery phase, in addition to a chalonic action, which increases the muscle size by inhibiting protein breakdown.In the present study urinary insulin was detected in equine samples and differences between equine insulin, human insulin, as well as rapidly acting recombinant insulin variants were examined. The method was based on sample purification by solid-phase extraction (SPE) and immunoaffinity chromatography (IAC), and subsequent analysis by microbore liquid chromatography (LC) and tandem mass spectrometry (MS/MS) using top-down sequencing for the determination of various insulins. Product ion scan experiments of intact proteins and B-chains enabled the differentiation between endogenously produced equine insulin, its DesB30 metabolite, human insulin and recombinant insulin analogs, and the assay allowed the assignment of individual product ions, especially those originating from modified C-termini of B-chains

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J Chromatogr A 2008 **1189** (1-2) 426
Comprehensive screening of acidic and neutral drugs in equine plasma by liquid chromatography-tandem mass spectrometry
Equine plasma was analysed for the purpose of doping control by development of a multi-target high-throughput liquid chromatography-tandem mass spectrometry (LC-MS-MS) method to detection low ppt to low ppb levels of anabolic steroids, corticosteroids, anti-diabetics, and non-steroidal anti-inflammatory drugs (NSAIDs). Deproteination of plasma samples was achieved by addition of trichloroacetic acid. Drugs were then isolated by solid-phase extraction (SPE) using Bond Elut Certify cartridges, and the extracts analysed with a triple-quadrupole/linear ion trap LC-MS-MS instrument in positive electrospray ionization (+ESI) mode with selected reaction monitoring (SRM) scan function. Chromatographic separation of the targeted drugs was achieved using a reverse phase 3.3 cm L x 2.1 mm ID, 3 µm particle size LC column with gradient elution. Plasma samples spiked with 66 targeted drugs including betamethasone, boldione, capsaicin, flunisolide, gestrinone, gliclazide, 17α-hydroxyprogesterone hexanoate, isoflupredone and triamcinolone acetonide, etc. at low ppt to low ppb levels could be routinely detected. No significant matrix interference was observed at the retention time of the targeted ion transitions when blank plasma samples were analysed. Extraction recoveries, precision and sensitivity of the technique was validated. This procedure is employed regularly in the authors' laboratory to screen for the presence of acidic and neutral drugs in plasma samples from racehorses by LC-MS-MS

8 Recreational Drugs - General

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Anal Bioanal Chem 2008 **392** (1-2) 115
Development and validation of a solid-phase extraction gas chromatography-mass spectrometry method for the simultaneous quantification of methadone, heroin, cocaine and metabolites in sweat
Methadone, heroin, cocaine and metabolites in sweat were simultaneously quantified with a sensitive and specific method. Drugs were extracted from sweat patches with sodium acetate buffer and subjected to SPE and with quantification by GC/MS using electron impact ionization and selected ion monitoring. Anhydroecgonine methyl ester, ecgonine methyl ester, cocaine, benzoylecgonine (BE), codeine, morphine, 6-acetylcodeine, 6-acetylmorphine (6AM), heroin (5-1000 ng/patch) and methadone (10-1000 ng/patch) were calibrated daily and resulted in determination coefficients of >0.995 with calibrators quantified to within +/-20% of the target concentrations. Extended calibration curves (1000-10,000 ng/patch) were produced for methadone, cocaine, BE and 6AM by modifying injection methods. Within (N = 5) and between-run (N = 20) imprecisions were calculated at six control levels across the dynamic ranges with coefficients of variation of <6.5%. At these concentrations, accuracies were +/-11.9% of target. Heroin hydrolysis during specimen processing was <11%. This novel assay offers effective monitoring of drug exposure during drug treatment, workplace and criminal justice monitoring programs

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Forensic Sci Int 2008 **177** (2-3) 105
Validation of an ion mobility spectrometry (IMS) method for the detection of heroin and cocaine on incriminated material
By means of a vacuum cleaner for sampling, the validation of a qualitative ion mobility spectrometry (IMS) procedure for the detection of trace amounts of heroin and cocaine on incriminated material is presented. The limit of detection, the limit of decision, selectivity and robustness were analysed. As an approach, robustness was determined using ionizational interferences and matrix effects. By employing this simple sampling procedure, a positive result for incriminated clothes requires a contamination of 250ng cocaine and 1000ng heroin, respectively

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Anal Bioanal Chem 2008 **392** (1-2) 105
Headspace sampling and detection of cocaine, MDMA, and marijuana *via* volatile markers in the presence of potential interferences by solid phase microextraction-ion mobility spectrometry (SPME-IMS)
Cocaine, methylenedioxymethylamphetamine (MDMA), and marijuana using SPME-IMS achieved by targeting their volatile markers (methyl benzoate, piperonal, and terpenes, respectively) were analyzed following successful air sampling. Routine methods of direct air sampling for drugs are ineffective because the parent compounds of these drugs have very low vapor pressures making them unsuitable for headspace sampling. Rather than targeting the parent drugs, IMS was operated at optimal settings (determined in previous work) in order to detect their volatile chemical markers. SPME is an effective and rapid air sampling method for the preconcentration of analytes which is particularly useful in confined spaces such as cargo containers where the volatile marker compounds of drugs may be found in adequate concentrations. After sampling the air using a 100 µm polydimethyl siloxane (PDMS) SPME fiber for as little as one minute, enough mass of the targeted volatile markers in the headspace of a quart-sized metal paint can (gallon, approximately 1101 cm^3) which contained sub-gram quantities of the drug samples was obtained for IMS detection. Further more, several potentially interfering compounds found in goods frequently shipped in cargo containers were tested individually as well as in mixtures with the drugs. Interferences with peaks were not observed for MDMA or marijuana, and minimal interferences were found for cocaine

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Drug Alcohol Depend 2008 **96** (3) 290
GC-MS confirmation of xylazine (Rompun), a veterinary sedative, in exchanged needles
The extent of xylazine (Xyz) abuse by injection in Puerto Rico was investigated. Two waves of used-syringe collections were performed. Firstly, syringes were gathered, anonymously and without additional information. Secondly, a short interview, also anonymous, was administered. We found Xyz in 37.6% of the collected syringes; the majority of the Xyz-containing syringes came from ranching communities. Syringes containing Xyz more frequently also contained "speedball" than those without (90.6% and 66.7%, respectively). Self-reports of Xyz injection deviated markedly from actual detection. Only 50% (self-described users) and 22% (self-described non-users) of the collected syringes contained the drug. Xyz users have a high prevalence of

skin ulcers (38.5% *vs*. 6.8%; p<0.001) and were more likely to be in poor health compared to non-users. Surprisingly, though a higher percentage of Xyz users than non-users had college-level educations (23.1% *vs*. 5.5%), they were more likely to be homeless (64.1% *vs*. 37%)

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J Chromatogr B 2008 **870** (2) 192
Integration of GC/EI-MS and GC/NCl-MS for simultaneous quantitative determination of opiates, amphetamines, MDMA, ketamine, and metabolites in human hair
Simultaneous hair testing of common drugs of abuse in Asia, including amphetamines (amphetamine, AP; methamphetamine, MA; methylenedioxy amphetamine, MDA; methylenedioxy methamphetamine, MDMA; methylenedioxy ethylamphetamine, MDEA), ketamine (ketamine, K; norketamine, NK), and opiates (morphine, MOR; codeine, COD; 6-acetylmorphine, 6-AM) was investigated using a multiple ionization mode approach of GC/MS. This technique combined the characteristics of gas chromatography-mass spectrometry (GC-MS) using electron impact ionization (EI) and negative chemical ionization (NCI). Hair samples (25 mg) were washed, cut, and incubated overnight at 25ºC in methanol-trifluoroacetic acid (methanol-TFA). The samples were extracted by solid phase extraction (SPE) procedure, derivatized using heptafluorobutyric acid anhydride (HFBA) at 70ºC for 30 min, and the derivatives analyzed by GC-MS with EI and NCI. The limit of detection (LOD) with GC/EI-MS analysis obtained were 0.03 ng/mg for AP, MA, MDA, MDMA, and MDEA; 0.05 ng/mg for K, NK, MOR, and COD; and 0.08 ng/mg for 6-AM. The LOD of GC/NCI-MS analysis was much lower than GC/EI-MS analysis. The LOD obtained were 30 pg/mg for AP and MDA in GC/EI-MS and 2 pg/mg in GC/NCI-MS. Clearly, the sensitivity of AP and MDA in GC/NCI-MS was improved 15-fold compared with EI. The sensitivity of AP, MA, MDA, MDMA, MDEA, MOR, and COD was improved from 15- to 60-fold compared with EI. In addition, the sensitivity of 6-AM increased 8-fold through selection of *m/z* 197 for the quantitative ion. Moreover, K and NK could dramatically improve their sensitivity at 200- and 2000-fold. The integration of GC/EI-MS and GC/NCI-MS may obtain the high sensitivity and complementary results of drugs of abuse in hair. Six hair samples from known drug abusers were invetigated by this new strategy. These results show that integrating the characteristics of GC/EI-MS and GC/NCI-MS resulted not only in the enhancement of the sensitivity but also avoids incorrect results and incorrect interpretations of correct results of these procedures

9 Stimulants

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J Chromatogr A 2008 **1183** (1-2) 21
Rapid screening of methamphetamines in human serum by headspace solid-phase microextraction using a dodecylsulfate-doped polypyrrole film coupled to ion mobility spectrometry
A simple, rapid and highly sensitive technique for simultaneous analysis of methamphetamine (MA) and 3,4-methylenedioxy methamphetamine (MDMA) in human serum is described using the solid-phase microextraction (SPME) combined with ion mobility spectrometry (IMS). A dodecylsulfate-doped polypyrrole (PPy-DS) is employed as a new fiber for SPME. Electrochemically polymerized PPy is formed on the surface of a platinum wire and contains charge-compensating anion (dodecylsulfate) incorporated during synthesis using cyclic voltammetry (CV) technique. The isolation characteristics of the fiber to MA and MDMA were assayed, using a headspace-SPME (HS-SPME) device and thermal desorption in injection port of IMS. The results indicate that PPy-DS as a SPME fiber coating is suitable for the successful isolation of these compounds. This technique is suitable for the identification and determination of MAs, is expeditious, necessitates small quantities of sample and does not require any derivatization. Parameters like pH, extraction time, ionic strength, and temperature of the sample were examined and optimized to obtain the best isolation results. The HS-SPME-IMS method produced good repeatability (RSDs<7.8 %) for spiked serum samples. The calibration graphs were linear in the range of 20-4000 ng ml^{-1} (R^2>0.99) and detection limits for MDMA and MA were 5 and 8 ng ml^{-1}, respectively. HS-SPME-IMS of non-spiked serum sample resulted in a spectrum without any peak from the matrix, supporting an effective sample clean-up. Finally, the proposed technique was employed for analysis of ecstasy tablet

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J Chromatogr B 2008 **861** (1) 22
Direct injection LC-MS/MS method for identification and quantification of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in urine drug testing
A method based on direct injection of diluted urine for the identification and quantification of amphetamine, methamphetamine, 3,4-methylenedioxymetamphetamine and 3,4-methylenedioxyamphetamine in human urine by electrospray ionisation liquid chromatography-tandem mass spectrometry was validated for use as a confirmation procedure in urine drug testing. Two deuterium labelled analogues, amphetamine-D5 and 3,4-methylenedioxymetamphetamine-D5, were employed as internal standards. Twenty microliter aliquots of urine were mixed with 80 µL internal standard solution in autosampler vials and 10 µL was injected. The chromatographic system consisted of a 2.0 mmx100 mm C_{18} column and the gradient elution buffers used acetonitrile and 25 mmol/L formic acid. Two product ions produced from the protonated molecules were monitored in the selected reaction monitoring mode. The intra- and inter-assay variability (coefficient of variation) was between 5 and 16% for all analytes at 200 and 6000 ng/mL levels. Ion suppression resulted soon after injection but did not affect the identification and quantification of the analytes in genuine urine samples. The method was further confirmed by comparison with a reference gas chromatographic-mass spectrometric method using 479 authentic urine samples. The two methods agreed almost completely (99.8%) regarding identified analytes when applying a 150 ng/mL reporting limit. Four deviating results were observed for 3,4-methylenedioxymethamphetamine and this resulted from uncertainty in quantification around the reporting limit. For the quantitative results the slope of the regression lines were between 0.9769 and 1.0146, with correlation coefficients >0.9339. We conclude that the liquid chromatographic-tandem mass spectrometric method described is robust and reliable, and suitable for use as a confirmation method in urine drug testing for amphetamines

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J Chromatogr A 2008 **1185** (1) 19
Simultaneous derivatization and extraction of amphetamine and methylenedioxyamphetamine in urine with headspace liquid-phase microextraction followed by gas chromatography-mass spectrometry
A new technique has been developed for the simultaneous extraction and derivatization of amphetamine (AM) and methylenedioxyamphetamine (MDA) using headspace hollow fiber protected liquid-phase microextraction (HS-HF-LPME); quantitation is by gas chromatograph-mass spectrometry in the selected ion monitoring (SIM) mode. The derivatizing reagent, pentafluorobenzaldehyde (PFBAY), was added to the extraction solvent. The analytes, volatile and basic, were released from the sample matrix into the headspace first, then isolated and derivatized in the solvent. Subsequently, 2 µl of extract was directly injected into the GC-MS system. Parameters affecting isolation efficiency were investigated and optimized. This technique provided good linearity in the concentration range employed (50-350 ng ml^{-1} for AM and 50-700 ng ml^{-1} for MDA). Excellent repeatability of the isolation (RSD< or = 4%, n=5), and low limits of quantitation (0.25 ng ml^{-1} for AM and 1.00 ng ml^{-1} for MDA) were achieved. The viability of the technique was illustrated by analysis of human urine samples

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J Chin Chem Soc 2008 **55** (3) 682
Influences of seven Taiwan-produced adulterants on gas chromatographic-mass spectrometric (GC-MS) urinalysis of amphetamines
The forensic gas chromatographic-mass spectrometric (GC-MS) confirmatory urinalysis of amphetamines was investigated to better understand the influences exerted by seven Taiwan-produced adulterants. The results verified that, when added at 5-15% (w/w), chlorine bleach resulted in lower GC-MS outcomes of the spiked and case specimens by 36-63%, and was most likely to cause false negatives. Liquid soap, potassium dichromate, soda water for drinking, and tap water resulted in a decrease of the GC-MS outcomes by 9-29%, 8-20%, 8-20%, and 5-16%, respectively, and possibly risked negating near-cutoff initial positives into false confirmatory negatives. The negative-directing effects were mostly due to degradation of analytes and/or deactivation of the derivatizing agent by oxidizing adulterants and/or dilution of analytes by the added liquid. Alum and table salt added as powder had little impact on the test. Investigating authorities should consider the results and include the specimen validity testing (SVT) battery in all of the routine drug testing procedures

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Forensic Sci Int 2008 **179** (2-3) 111
Headspace profiling of cocaine samples for intelligence purposes
Residual solvents in illicit hydrochloride cocaine samples were investigated using static headspace-gas chromatography (HS-GC) associated with a storage computerized procedure for the profiling and comparison of seizures. The technique involves a gas chromatographic separation of 18 occluded solvents followed by fully automatic data analysis and transfer to a PHP/MySQL database. Firstly, a fractional factorial design was employed to evaluate the main effects of some critical method parameters (salt choice, vial agitation intensity, oven temperature, pressurization and loop equilibration) on the results with a minimum of experiments. The technique was then validated for tactical intelligence purposes (batch comparison) *via* several studies: selection of solvents and mathematical comparison tool, reproducibility and "cutting" influence studies. The decision threshold to determine the similarity of two samples was set and false positives and negatives evaluated. Finally, application of the technique to identify geographical origins is discussed

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J Chromatogr B 2008 **862** (1-2) 252

Designer drug 2,5-dimethoxy-4-methyl-amphetamine (DOM, STP): Involvement of the cytochrome *P*450 isoenzymes in formation of its main metabolite and detection of the latter in rat urine as proof of a drug intake using gas chromatography-mass spectrometry

The designer drug 2,5-dimethoxy-4-methyl-amphetamine (DOM, STP) is known to be extensively metabolized in various species. The current study demonstrated that cytochrome *P*450 2D6 was the only isoenzyme involved in the formation of the main metabolite hydroxy DOM. Furthermore, the authors' systematic toxicological analysis (STA) procedure using full-scan GC-MS was applicable to demonstrate consumption of a common drug users' dose of DOM by detection of hydroxy DOM in rat urine. Assuming similar metabolism, the described STA technique should be appropriate for proof of an intake of DOM in human urine. However, DOM and/or other metabolites such as deamino-oxo-hydroxy DOM might be the target analyte in urine of CYP2D6 poor metabolizers

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J Chromatogr B 2008 **867** (2) 194

Development and validation of a liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry method for simultaneous analysis of 10 amphetamine-, methamphetamine- and 3,4-methylenedioxymethamphetamine-related (MDMA) analytes in human meconium

A liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (LC-APCI-MS/MS) technique for quantification of 10 amphetamine-related analytes in 1g meconium has been developed. Specimen preparation included homogenization and solid-phase extraction. Two multiple reaction monitoring transitions were monitored per analyte. Ten and 1 µL injection volumes permitted quantification up to 10,000 ng/g, with sufficient sensitivity to quantify minor metabolites. Lower limits of quantification ranged from 1.25 to 40 ng/g. Precision was less than 14.2%, with accuracy between 79 and 115%. Meconium from a methamphetamine-exposed neonate was analyzed. Metabolites p-hydroxymethamphetamine, norephedrine and 4-hydroxy-3-methoxymethamphetamine were identified in meconium for the first time

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J Chromatogr B 2008 **867** (1) 78

Analysis of amphetamine-type stimulants and their metabolites in plasma, urine and bile by liquid chromatography with a strong cation-exchange column-tandem mass spectrometry

This work was intended to develop and validate a method for analysing amphetamine-type stimulants (ATSs) and their metabolites in plasma, urine and bile by liquid chromatography with a strong cation-exchange column-tandem mass spectrometry, and to apply it to the pharmacokinetic study of ATSs. 3,4-Methylenedioxymethamphetamine, methamphetamine, ketamine and their main metabolites, 4-hydroxy-3-methoxymethamphetamine, 3,4-methylenedioxyamphetamine, *p*-hydroxymethamphetamine, amphetamine and norketamine, were simultaneously quantified by the new method (50-5000 ng/ml). The coefficients of variation and the percent deviations for the eight compounds were in the range of 0.2 to 5.3% and -9.4 to +12.8%, respectively. The recoveries were over 90% in all biological samples tested. This technique was effective for the separation and the identification of ATSs and their main metabolites having amine moieties in plasma, urine and bile. It was applicable to pharmacokinetic analysis of methamphetamine, ketamine and their main metabolites in biological samples. This analytical technique should be useful for the pharmacokinetic analysis of ATSs

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Anal Chim Acta 2008 **619** (1) 20

Determination of impurities in illicit methamphetamine seized in Korea and Japan

Methamphetamine (MA) exhibits different patterns of impurities under various synthetic conditions. Important information on the source and smuggling routes may be produced by using impurities as chemical fingerprints. More than 100 compounds from 436 MA samples we detected in seized goods in Korea by gas chromatography-flame ionization detector and gas chromatography-mass spectrometer, among which 31 impurities and three additives were identified. Twenty-six impurity peaks including unknowns were identified as the indicators of similarity and were used as variables for cluster analysis. This showed that part of the MA samples seized in Japan might have the same origin as those seized in Korea. It suggests that broad-based cooperation is necessary for efficient regulation of MA. Synthetic trends of the MA seizures of Korea were monitored by cluster analysis with 16 MA samples synthesized by three different methods in the previous work. Changes of synthetic trends, which might have been influenced by domestic regulations and international situations, were identified

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J Chromatogr B 2008 **865** (1-2) 25

Development of a two-step injector for GC-MS with on-column derivatization, and its application to the determination of amphetamine-type stimulants (ATS) in biological specimens

A two-step auto-injector for the automated on-column derivatization and subsequent GC-MS of amine-type drugs and metabolites is described. To effectively derivatize such analytes, this injector has been designed to inject the derivatization reagent several seconds after the sample has been injected. Eleven types of amphetamine-type stimulants (ATS) and their typical metabolites were analysed using the trifluoroacetylation reagent *N*-methyl bis(trifluoroacetamide) (MBTFA). Although the quantitative derivatization of the hydroxyl groups was difficult, this technique was successfully applied to the determination of ATS in urine, blood, and hair specimens. The limits of detection for methamphetamine and amphetamine in hair were 0.2 and 0.1 ng/mg hair respectively in the full-scan mode when a 10 mg hair sample was analyzed

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J Chromatogr B 2008 **867** (1) 126

LC-MS analysis of trimethoxyamphetamine designer drugs (TMA series) from urine samples

A sensitive liquid chromatography-mass spectrometric (LC-MS) method was developed and validated for quantification of an active psychedelic hallucinogenic drugs (trimethoxyamphetamines) in human urine after solid-phase extraction (SPE) with C_{18} cartridge. Chromatographic separation was achieved on reversed-phase Phenomenex 3.0 µm Polar Plus column (150 mm x 2.1 mm) with acetonitrile -0.2% acetic acid as mobile-phase and the step gradient elution resulted in a total run time of about 20 min. The analytes were detected by using an electrospray positive ionization mass spectrometry in selected ion monitoring (SIM) mode. In the evaluated concentration range (10-200 ng/mL) (R^2 > or = 0.998) a good linear relationship was obtained. The lower limits of detection (LLODs) and quantification (LLOQs) ranged from 4.26 to 9.12 ng/mL and from 13.18 to 29.22 ng/mL, respectively. Average recoveries ranged from 68.52 to 97.90% in urine at the concentrations of 25, 50 and 100 ng/mL. Intra- and inter-day relative standard deviations were 3.70-10.77% and 7.63-12.94%, respectively. This LC-MS method proved to be robust and reliable, and suitable for the use as a confirmation method in clinical urine drug testing

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Spectrosc Lett 2008 **41** (3) 101

Assignments of ^{1}H and ^{13}C NMR spectral data for benzoylecgonine, a cocaine metabolite

The complete assignment of the ^{1}H and ^{13}C NMR spectra of benzoylecgonine, a cocaine metabolite, was investigated employing 2D experiments such as gCOSY and gHSQC

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Anal Chim Acta 2008 **606** (2) 217

Parallel analysis of stimulants in saliva and urine by gas chromatography/mass spectrometry: Perspectives for "in competition" anti-doping analysis

Stimulants are banned by the World Anti-Doping Agency (WADA) if used "in competition". Since analysis is currently carried out on urine samples only, it would be helpful to discriminate between early intake of the substance and administration specifically intended to improve sports performance. The purpose of the study was to investigate the differences, in terms of excretion/disappearance of drugs, between urine and oral fluid. Oral fluid and urine samples were obtained after oral administration of the following stimulants: modafinil (100 mg), selegiline (10 mg), crotetamide/cropropamide (50 mg each), pentetrazol (100 mg), ephedrine (12 mg), sibutramine (10 mg), mate de coca (a dose containing about 3mg of cocaine); analysis of drugs/metabolites was carried out by GC/MS for both body fluids. The results show that both the absolute concentrations and their variation as a function of time, in urine and in oral fluid were generally markedly different. The drugs were eliminated from urine much more slowly than from oral fluid. The results also suggest that the analysis of oral fluid could be used to successfully complement the data obtained from urine for "in competition" anti-doping tests. Particularly, in all those cases where the metabolite(s) concentration of a substance in urine was very low and the parent compound was not detected. It was impossible to discriminate between recent administrations of small doses and older administration of higher doses when relying on urinary data only

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Biosens Bioelectron 2008 **24** (1) 111

Development of a bifunctional sensor using haptenized acetylcholinesterase and application for the detection of cocaine and organophosphates

A dual piezoelectric/amperometric sensor was developed for the detection of two unrelated analytes in one experiment that uses propidium to anchor acetylcholinesterases (AChE) at the surface. This mass-sensitive sensor does not only allow the examination of the interaction between AChE and the modified surface but also the detection of in situ inhibition of the surface-bound AChE. Herein is described the application of the propidium-based sensor in combination with modified AChE. For this reason the cocaine derivative benzoylecgonine (BZE) was coupled *via* a 10A long hydrophilic linker - 1,8-diamino-3,4-dioxaoctane - to carboxylic groups of the AChE after EDC/NHS activation. Thus the modified AChE (BZE-AChE) possesses an additional recognition element besides the inhibitor binding site. After the deposition of BZE-AChE on the sensor surface the binding of an anti-BZE-antibody to the BZE-AChE may be monitored. This makes it possible to determine two analytes - cocaine and organophosphate - in one experiment by measuring antibody binding and decrease in enzymatic activity, respectively. in addition, it was also shown that other cocaine-binding enzymes, e.g., butyrylcholinesterase, may bind to the modified BZE-AChE. The competitive immunoassay allowed the detection of cocaine with a dynamic range from 10^{-9} to 10^{-7}M. The organophosphate chlorpyrifos-oxon could be detected in concentrations from 10^{-6} down to 10^{-8}M after 20 min of injection time (equals to 500 µL sample volume

10 Hallucinogens

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Forensic Sci Int 2008 **178** (1) 61

GC-MS studies on acylated derivatives of 3-methoxy-4-methyl- and 4-methoxy-3-methyl-phenethylamines: Regioisomers related to 3,4-MDMA

A series of side chain regioisomers of 3-methoxy-4-methyl- and 4-methoxy-4-methyl-phenethylamines have mass spectra essentially equivalent to the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA), all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. The acetyl, propionyl and trifluoroacetyl derivatives of the primary and secondary regioisomeric amines were prepared and analysed by GC-MS. The mass spectra for these derivatives were significantly individualized and the resulting unique fragment ions allowed for specific side chain identification. The trifluoroacetyl derivatives resulted in more fragment ions for molecular individualization among these regioisomeric substances. Trifluoroacetyl derivatives showed excellent resolution on a non-polar stationary phase such as Rtx-1

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J Anal Toxicol 2008 **32** (3) 227

Development and clinical application of an LC-MS-MS method for mescaline in urine

Mescaline (3,4,5-trimethoxyphenylethylamine) is an hallucinogenic psychoactive substance present in several species of cacti and has a documented use dating back 5700 years. Recently, the interest in hallucinogenic designer drugs such as ecstasy has also resulted in interest in the naturally occurring mescaline. This study was undertaken to produce a liquid chromatography-tandem mass spectrometry (LC-MS-MS) technique for the screening and confirmation of mescaline in human urine samples and to apply this method to routine testing in patient samples. For the screening procedure, chromatographic separation was achieved on a 5-µm HyPURITY C_{18} column, using a methanol gradient in ammonium acetate buffer. The MS-MS analysis was performed using selected reaction monitoring; the transitions monitored were *m/z* 212.3 → *m/z* 180.3 for mescaline and *m/z* 221.3 → *m/z* 186.3 for the deuterated internal standard (mescaline-d_9). The detection limit for mescaline in urine matrix was 3-5 µg/L, the upper limit of quantification was 10,000 µg/L, and the total coefficient of variation for spiked samples containing 10 to 1025 µg/L was < 8.5%. The confirmation procedure included a sample clean-up by solid-phase extraction on a C_{18} cartridge, and one extra transition for mescaline (*m/z* 212.3 → *m/z* 195.2) was monitored. The LC-MS-MS technique was found to be sensitive and specific for the routine detection of mescaline in urine. Of 462 urine samples collected from young people with alcohol or drug problems, 32% were positive for illicit drugs, but none for mescaline

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Acta Clin Belg 2008 **63** (3) 200

-Hydroxybutyric acid (GHB) measurement by GC-MS in blood: Urine and gastric contents following an acute intoxication in Belgium

γ-Hydroxybutyrate (GHB, sodium oxybate) is a compound related to neuromodulator γ-aminobutyric acid (GABA). Recently, it has featured as a recreational drug of abuse and as a rape drug. GHB-related emergencies have dramatically increased in the 1990s. However, a decrease has been observed since 2000. A case is described of acute GHB intoxication in a 28-year-old male who fell unconscious following consumption of a mouthful of an unknown beverage. Subsequently, he required medical support for 2 days. Cocaine abuse was detected by preliminary toxicological screening but the clinical presentation was atypical of cocaine intoxication. A simple liquid-liquid extraction was employed for quantitation of GHB, followed by disilyl-derivatization and analysis in selective ion monitoring (SIM) mode by gas chromatography-mass spectrometry (GC-MS), using GHB-d6 as internal standard. High concentrations of GHB were detected in urine (3020 mg/L) and gastric contents (71487 mg/L) at admission. Following a period of 6-hours, GHB was identifiable in urine at 2324 mg/L and in blood at 43 mg/L. The clinical symptoms of cocaine intoxication were diminished by GHB consumption, and the cerebral scan was modified. Acute intoxications with unusual clinical symptoms require investigation. Consequently, GHB should be added to the preliminary toxicological screening. Data available regarding GHB are briefly reviewed and our results are compared with previously published reports of non-fatal GHB intoxication

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J Chromatogr B 2008 **863** (1) 123

Detection of phencyclidine in human oral extraction and liquid chromatography fluid using solid-phase with tandem mass spectrometric detection

An analytical method for the determination of phencyclidine in oral fluid has been produced and validated using liquid chromatography with tandem mass spectral detection, following initial screening with enzyme linked immunosorbent assay. The oral fluid samples were collected using the Quantisal device and any drugs present were quantified using mixed mode solid-phase extraction followed by mass spectrometric detection in positive atmospheric pressure chemical ionization mode. For confirmation, two transitions were monitored and one ratio determined, which had to be within 20% of that of the known calibration standard. The monitoring of the qualifying transition and requirement for its presence within a specific ratio to the primary ion has the potential of limiting the sensitivity of the assay. However, the additional confidence in the final result as well as forensic defensibility were considered to be of greater importance. The limit of quantitation was 5ng/mL; the intra-day precision of the assay (n=5) was 3.04%; inter-day precision 3.35% (n=5) at a concentration of 10ng/mL. The accuracy was determined at four concentrations (5, 10, 20 and 40ng/mL) within the linear range of the assay. The percentage isolation of phencyclidine from the oral fluid collection pad was 81.7% (n=6). The techniques were applied to both proficiency specimens and to samples obtained during research studies carried out in the USA

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Clin Chem 2008 **54** (2) 379

Two-dimensional gas chromatography/electron-impact mass spectrometry with cyclofocusing for simultaneous quantification of MDMA, MDA, HMMA, HMA and MDEA in human plasma

BACKGROUND: 3,4-Methylenedioxymethamphetamine (MDMA, or Ecstasy) is a popular recreational drug. Analysis of MDMA and metabolites in human plasma, especially in pharmacokinetic studies, necessitates low limits of quantification. Two-dimensional GC/MS with cryofocusing is a chromatographic technique known for its enhanced selectivity and resolution. METHODS: The method described simultaneously quantifies 3,4-methylenedioxyethylamphetamine (MDEA), MDMA, and its metabolites, 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and 4-hydroxy-3-methoxyamphetamine (HMA) in human plasma. Using hydrochloric acid, we hydrolyzed 1 mL plasma, containing the internal standard. Analytes were subjected to solid-phase extraction, derivatized with heptafluorobutyric acid anhydride, and quantified using cryofocused 2-dimensional GC/MS operated in electron-impact selected ion-monitoring mode. RESULTS: Limits of quantification were 1.0 µg/L for MDA and 2.5 µg/L for MDEA, MDMA, HMMA, and HMA. Calibration curves were linear to 100 µg/L for MDA and HMA and to 400 µg/L for MDEA, MDMA, and HMMA, with $r^2 > 0.997$. At 3 concentrations across the linear dynamic range of the assay, mean overall isolation efficiencies from plasma were > or =85% for all compounds of interest. Recoveries were 85.6% to 107.2% of target, and intra- and interassay imprecision (CV) was <8.5% for all drugs at 3 concentrations within the range of the assay.Of 66 exogenous compounds tested, none interfered with analyte quantification. CONCLUSIONS: The GC/MS assay described provides low limits of quantification for simultaneous determination of MDEA, MDMA, and metabolites MDA, HMMA, and HMA in human plasma. The 2D chromatographic system should be suitable for application to other analytes and to other complex matrices

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J Chromatogr A 2008 **1204** (1) 87

Validated gas chromatographic-mass spectrometric analysis of urinary cannabinoids purified with a calcium-hardened -cyclodextrin polymer
Extraction of cannabinoids following an inclusion complex between β-cyclodextrin (βCD) and cannabinoids including Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) by solid-phase extraction (SPE) is described for gas chromatographic-mass spectrometric (GC-MS) analysis. A βCD/epichlorohydrin copolymer was prepared and then 'hardened' in aqueous solution with 0.3M $CaCl_2$ to yield a stable particulate copolymer, which was used as a novel SPE sorbent. An internal standard THC-COOH-d_9 was added to urine samples containing 3 cannabinoids and then purified with the hardened βCD polymer. The cannabinoids were isolated from the hardened βCD using tetrahydrofuran. Resulting extracts were evaporated and derivatized with MSTFA/NH_4I/dithioerythritol (500:4:2, v/w/w) and analyzed by GC-MS in selected-ion monitoring (SIM) mode. Overall recoveries ranged from 85% to 102%, with a detection limit of 0.2 μg L^{-1} for the three cannabinoids tested. The precision (% CV) and accuracy (% bias) of the assay were 1.2-5.1% and 93-111% in 0.2-50 μg L^{-1} calibration range, respectively (r^2>0.9997). Twenty genuine samples which were positive by fluorescence polarization immunoassay were also quantitatively investigated. The method based on the calcium-hardened βCD sorption of cannabinoids and subsequent GC-SIM/MS demonstrated better selectivity and extraction efficiency than is possible using the conventional hydrophobicity-based SPE methods

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J Pharm Biomed Anal 2008 **47** (2) 335
Liquid chromatography-atmospheric pressure ionization electrospray mass spectrometry determination of "hallucinogenic designer drugs" in urine of consumers
A liquid chromatography-mass spectrometry (LC-MS) based technique has been developed for determination of 3,4-methylenedioxymethamphetamine (MDMA), 2,5-dimethoxy-4-methyl-phenethylamine (2C-D), 4-bromo-2,5-dimethoxy-β-phenethylamine (2C-B), 1-(8-bromo-2,3,6,7-tetrahydrobenzo [1,2-b:4,5-b'] difuran-4-yl)-2-aminoethane (2C-B-Fly), 4-ethylthio-2,5-dimethoxy-β-phenethylamine (2C-T-2), 4-iodo-2,5-dimethoxy-β-phenethylamine (2C-I), and 4-ethyl-2,5-dimethoxy-β-phenethylamine (2C-E), 1-(*m*-chlorophenyl)piperazine (*m*-CPP), 4-hydroxy-*N,N*-diisopropyltryptamine (4-OH-DIPT) and 4-acetoxy-*N,N*-diisopropyltryptamine (4-acetoxy-DIPT) in urine of consumers using 3,4 methylendioxypropylamphetamine (MDPA) as internal standard. Sample preparation involved a solid-phase extraction procedure at pH 6 of both non-hydrolyzed and enzymatically hydrolyzed urine samples. Chromatography was performed on a C_{18} reversed-phase column using a linear gradient of 10mM ammonium bicarbonate, pH 7.3 and acetonitrile as a mobile phase. Analytes were separated and determined in LC-MS single ion monitoring mode using an atmospheric pressure ionization-electrospray ionization (ESI) interface. The assay was tested on urine samples from consumers of compounds under investigation (n=32). Limits of quantification varied between 20 and 60 ng/mL for the different analytes under investigation. Calibration curves were linear to 2000 ng/mL for all the substances under investigation, with a minimum r^2>0.99. At three concentrations spanning the linear dynamic range of the assay, mean recoveries ranged between 55.4 and 95.6% for the different analytes. Higher concentrations of analytes in hydrolyzed samples showed the presence of conjugated compounds in urine

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J Chromatogr A 2008 **1186** (1-2) 380
Metabolism and toxicological detection of a new designer drug, *N*-(1-phenylcyclohexyl) propanamine, in rat urine using gas chromatography-mass spectrometry
Methods are described to study the metabolism and the toxicological detection of the phencyclidine-derived designer drug *N*-(1-phenylcyclohexyl)-propanamine (PCPR) in rat urine using gas chromatographic-mass spectrometry . Metabolites indicate that PCPR was metabolized by hydroxylation of the cyclohexyl ring at different positions, hydroxylation of the phenyl ring, *N*-dealkylation, and combinations of these steps. Parts of the metabolites were excreted in conjugated form. The authors' systematic toxicological analysis (STA) procedure using full-scan GC-MS after acid hydrolysis, liquid-liquid extraction and microwave-assisted acetylation allowed the detection of consumption of a common drug users' dose of PCPR in rat urine. If similar metabolism occurs in humans, the STA should be applicable to verify consumption of PCPR *via* human urine

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J Chromatogr B 2008 **871** (1) 101
Accurate identification and quantification of 11-nor- ^{9}-tetrahydrocannabinol-9-carboxylic acid in urine drug testing: Evaluation of a direct high efficiency liquid chromatographic-mass spectrometric method
A technique for measurement of urinary Δ^9-tetrahydrocannabinol carboxylic acid (THCA) was developed employing direct liquid chromatographic-tandem mass spectrometric (LC-MS/MS). The technique involved dilution of the urine sample with water containing $^2H_9$-deuterated analogue as internal standard, hydrolysis with ammonia, reversed phase chromatography using a Waters ultra-performance liquid chromatography (UPLC) equipment with gradient elution, negative electrospray ionization, and monitoring of two product ions in selected reaction monitoring mode. The measuring range was 2-1000 ng/mL for THCA, and the intra- and inter-assay imprecision, expressed as the coefficient of variation, was below 5%. Influence from urine matrix on ionization efficiency was noted in infusion experiments, but was compensated for by the internal standard. Following comparison with established gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry methods in authentic patient samples accuracy was demonstrated in both qualitative and quantitative results. A small difference in mean ratios (~15%) may result from the use of different hydrolysis procedures between methods. Finally, the high efficiency LC-MS/MS method was capable of accurately identify and quantify THCA in urine with a capacity of 14 samples per hour

11 Narcotics

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J Anal Toxicol 2008 **32** (3) 253
Simultaneous determination of ketamine, tramadol, methadone, and their metabolites in urine by gas chromatography-mass spectrometry
The identification and quantitation of ketamine, norketamine, tramadol, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, and 2-ethyl-5-methyl-3,3-diphenylpyrroline in urine by an automated solid-phase extraction procedure combined with gas chromatography-mass spectrometry methodology, without derivatization is described. The analytical technique is simple and rapid, yet reliable. Good linearity (r^2 > 0.995 over the concentration range of 30 to 600 ng/mL), sensitivity (limits of quantitation 15-30 ng/mL), accuracy (81.0-109.9%), precision (RSD < 13.8%), and recovery (> 79.6% in average) were achieved for all analytes. Ninety-one urine specimens from suspected drug users and 21 clinical urine specimens from methadone substitution therapy patients were analyzed to confirm the method compatibility and stability. Results have illustrated that this GC-MS method is a good confirmation and quantitation test scheme for the six target compounds in urine

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Anal Bioanal Chem 2008 **392** (1-2) 215
Group-selective antibodies based fluorescence immunoassay for monitoring opiate drugs
Polyclonal antibodies against the target molecule heroin and its major metabolites were generated with a novel carboxylic acid derivative of monoacetylmorphine (MAM-COOH) which was synthesized and conjugated with bovine serum albumin (BSA). The conjugate was characterized by fluorescence spectroscopy, polyacrylamide gel electrophoresis, and mass spectrometry to confirm the extent of haptenization of the carrier protein. A high titer (1:64,0000) of antibody was obtained by using the conjugate with an optimum protein/hapten molar ratio of 1:100. The generated antibody showed good binding affinity with heroin and its metabolites monoacetylmorphine (MAM) and morphine. The relative affinity constant (K_{aff}) of the antibody was 3.1 x 10^7 l mol^{-1}, and the IC_{50} values obtained for heroin, MAM, morphine, and codeine were 0.01, 0.013, 0.012, and 0.014 ng ml^{-1}, respectively. A fluorescence-based competitive inhibition immunoassay technique is described for the analysis of heroin and its major metabolites in standard and biofludic samples over a concentration range up to 0.01 ng ml^{-1} with good signal reproducibility ($p < 0.05$). This technique may be used as a convenient quantitative tool for the sensitive screening of major metabolites of heroin in biological samples

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Microchem J 2008 **89** (2) 137
Simultaneous determination of pethidine and methadone by capillary electrophoresis with electrochemiluminescence detection of tris(2,2'-bipyridyl)ruthenium(II)
Capillary electrophoresis with electrochemiluminescence (CE–ECL) detection using tris(2,2'-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$), to simultaneously detect pethidine and methadone is described. Analytes were injected into a separation capillary of 67.5 cm length (25 μm i.d., 360 μm o.d.) by electrokinetic injection for 10 s at 10 kV. Under the optimized conditions: ECL detection at 1.20 V, 30 mM sodium phosphate (pH 6.0) as running buffer, separation voltage at 14.0 kV, 5 mM $Ru(bpy)_3^{2+}$ with 50 mM sodium phosphate (pH 6.5) in the detection cell, the linear range from 2.0 × 10^{-6} to 2.0 × 10^{-5} M for pethidine and 5.0 × 10^{-6} to 2.0 × 10^{-4} M for methadone and detection limits of 0.5 μM for both of them were achieved (S/N = 3). Relative SDs of the ECL intensity were 2.09% for pethidine and 6.59% for methadone

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Forensic Sci Int 2008 **174** (2-3) 197
Analysis of ketamine and norketamine in urine by automatic solid-phase extraction (SPE) and positive ion chemical ionization-gas chromatography-mass spectrometry (PCI-GC-MS)
Ketamine (KT) is widely abused for hallucination and also misused as a "date-rape" drug in recent years. An analytical method using positive ion chemical ionization-gas chromatography-mass spectrometry (PCI-GC-MS) with an automatic solid-phase extraction (SPE) apparatus was employed for the determination of KT and its major metabolite (norketamine - NK), in urine. Six urine samples were provided by the police in ketamine suspected cases. KT metabolism was investigated by administration to SD rats by i.p. at a single dose of 5, 10 and 20mg/kg, respectively. Urine samples were collected 24, 48 and 72 h after administration. For the detection of KT and NK, urine samples were extracted on an automatic SPE apparatus (RapidTrace, Zymark) with mixed mode type cartridge, Drug-Clean (200 mg, Alltech). Calibration curves were linear from 50 to 1000 ng/mL for KT and NK with correlation coefficients in excess of 0.99. The limit of detection (LOD) was 25 ng/mL for KT and NK. The limit of quantitation (LOQ) was 50 ng/mL for KT and NK. Recoveries of KT and NK at three different concentrations (86, 430 and 860 ng/mL) were 53.1 to 79.7% and 45.7 to 83.0%, respectively. The intra- and inter-day run precisions (CV) for KT and NK were less than 15.0%, and the accuracies (bias) for KT and NK were also less than 15% at the three different concentration levels (86, 430 and 860 ng/mL). Six KT suspect urine specimens and KT administered rat urines were anlysed and the concentrations of KT and NK were determined. Dehydronorketamine (DHNK) was also confirmed in these urine samples. However the concentration of DHNK was not calculated. SPE is simple and needs less organic solvent than liquid-liquid extraction (LLE). PCI-GC-MS offers both qualitative and quantitative information for analysis of KT in urine of forensic analysis

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J Chromatogr A 2008 **1188** (2) 322
Applicability of ultra-performance liquid chromatography-tandem mass spectrometry for heroin profiling
Ultra-performance liquid chromatography tandem-mass spectrometry (UPLC-MS/MS) for heroin profiling is described. The coupling of the high separation power of UPLC with the highly selective and sensitive detection of MS/MS is well suited for heroin analysis. An Acquity UPLC BEH C18 1.7 µm particle column (100 mm x 2.1mm) with binary gradients containing 1% formic acid (pH 2.0) or 10 mM ammonium bicarbonate (pH 10.0)/acetonitrile mixtures was investigated for analysis. For MS/MS detection, an atmospheric pressure positive electrospray source was employed with multiple reaction monitoring (MRM). MRMs for individual basic impurities were generated for heroin profiling using low and high pH mobile phases, while MRMs for neutral impurities were produced with a high pH mobile phase. Compared to a pH 2.2 mobile phase, the use of a pH 10 mobile phase enabled significantly greater sample loading, major selectivity differences, and lower MRM sensitivity. UPLC-MS/MS provided highly selective and sensitive detection of many of the targeted solutes in seized heroin exhibits. Basic impurities detected included morphine, codeine, noscapine, papaverine and the previously unreported solutes reticuline, reticuline monoacetate (2 products), reticuline diacetate, narceine, codamine, laudanidine, cryptopine, laudanosine, and norlaudanosine. Neutral impurities found included *N*,3,6-triacetylnormorphine, *N*-acetylnorcodeine, *N*-acetylnornarcotine, 3,6-dimethoxy-4-acetyloxy-5-[2-(*N*-methylacetamido)]-ethylphenanthrene, and *cis*-*n*-acetylanhydronornarceine. Greatly enhanced heroin analysis with levels of detection of impurities as low as 10^{-6}% w/w is achievable

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J Anal Toxicol 2008 **32** (3) 208
An enantiomer-selective liquid chromatography-tandem mass spectrometry method for methadone and EDDP validated for use in human plasma, urine, and liver microsomes
Detection (*R*)- and (*S*)-methadone and (*R*)- and (*S*)-2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in human plasma with cross-validation to urine and liver microsomes was achieved by development and validation of a liquid chromatography-electrospray ionization-tandem mass spectrometry technique. Deuterated internal standards and liquid-liquid extraction coupled with chiral separation allowed baseline separation with a lower limit of quantitation (LLOQ) of 2.5 ng/mL. The LLOQ was established from comparison of signal in blanks from six different sources per matrix with the same sources fortified at the LLOQ (none exceeded 19% of LLOQ) and precision and accuracy at the LLOQ determined in the same six sources per matrix. The assay was precise (% coefficients of variation within 13.8%) and accurate (% targets within 15%) in all three matrices. The addition of other psychoactive drugs resulted in no interference. Stability was determined in plasma (24 h at room temperature, 321 days at -20ºC, 3 freeze-thaw cycles); processed plasma samples (5 days at -20ºC, 12 days on autosampler); urine (24 h at room temperature); and stock solutions (20 h at room temperature, 61 days at -20ºC). Applications of varying degree are presented for each matrix. Plasma from five subjects maintained on 100 mg oral methadone per day allowed comparison of the pharmacokinetics of the enantiomers. The $t_{1/2}$ of (*R*)-methadone was significantly longer than for (*S*)-methadone, and (*S*)-methadone was more tightly protein bound. The C_{max}, AUC, C_{min}, and % protein bound of (*S*)-EDDP were significantly greater than (*R*)-EDDP, while the $t_{1/2}$ of (*R*)-EDDP was significantly greater than (*S*)-EDDP. In spot urines, (*R*)- was higher than (S)-methadone, and (*S*)- was generally higher than (*R*)-EDDP. (*R*)- and (*S*)-EDDP production was detected after incubation of therapeutic concentrations of racemic methadone with human liver microsomes, and (*S*)-EDDP production was twofold greater than (*R*)-EDDP in three human placental microsomes incubated with racemic methadone

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J Chromatogr B 2008 **867** (2) 219
Validated method for the simultaneous determination of methadone and its main metabolites (EDDP and EMDP) in plasma of umbilical cord blood by gas chromatography-mass spectrometry
The determination of methadone (MDN) and its two main metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) in plasma samples obtained from venous and arterial umbilical cord blood and maternal blood has been obtained following the develoment, optimization and validation of a sensitive and specific GC/MS method. Specimen preparation included protein precipitation with acetonitrile and simultaneous solid-phase extraction of the three analytes. Methadone-d_9 was used as internal standard for the determination of MDN and EMDP, while EDDP-d_3 for EDDP. Limits of detection were 0.6 µg/L for MDN and 0.3 µg/L for EDDP and EMDP, while limits of quantification were 2.0 µg/L for MDN and 1.0 µg/L for EDDP and EMDP. The calibration curves were linear up to 2000 µg/L for MDN and up to 1000 µg/L for EDDP and EMDP. Absolute recovery ranged from 94.8 to 99.7% for all three analytes. Intra-and interday accuracy was less than 5.3 and 5.5%, respectively, while intra- and interday precision was less than 3.5 and 5.0%, correspondingly, for all analytes. The method proved suitable for the determination of MDN and its two main metabolites in plasma samples obtained from umbilical cord and maternal blood of a woman participating in a MDN maintenance program, during the prenatal and postpartum period

Twigger S, Fox E, Allen K// Leeds Teaching Hosp, Dept Clin Biochem, Leeds LS27 0DQ, England
Ann Clin Biochem 2008 **45** (3) 339
Measurement of buprenorphine in urine: Immunoassay *versus* LC-MS/MS (Letter)
No abstract available

12 Forensics

Cheze M, Lenoan A, Deveaux M, Pepin G// Lab TOXLAB, 7 rue Jacques Cartier, FR-75018 Paris, France
Forensic Sci Int 2008 **176** (1) 58
Determination of ibogaine and noribogaine in biological fluids and hair by LC-MS/MS after *Tabernanthe iboga* abuse iboga alkaloids distribution in a drowning death case
Tabernanthe iboga belongs to the Apocynaceae family. The case of a 37-year-old black male working as a security agent in Paris and found dead naked on the beach in Gabon after consumption of iboga is reported. Autopsy revealed a drowning fatality and a myocardial abnormality (myocardial bridging). Samples of blood, urine, bile, gastric content, liver, lungs, vitreous, spleen and hair were obtained. Biological fluids were liquid-liquid extracted with saturated NH_4Cl pH 9.5 and methylene chloride/isopropanol (95/5, v/v) in presence of clonazepam-d_4, employed as internal standard. Following decontamination with dichloromethane, hair was cut into small pieces then sonicated for 2h in saturated NH_4Cl pH 9.5 before extraction by methylene chloride/isopropanol (95/5, v/v). After evaporation the residues were reconstituted in methanol/ACN/formate buffer pH 3, from which 10 µL were injected into an ODB Uptisphere C_{18} column (150 mm x 2.1mm, 5 µm) and eluted with a gradient of acetonitrile and formate buffer delivered at a flow rate of 200 µL/min. A Quantum Ultra triple-quadrupole mass spectrometer was employed for analyses. Ionization was achieved using electrospray in the positive ionization mode (ESI). For each compound, detection was related to three daughter ions (ibogaine: *m/z* 311.4→122.1, 174.1 and 188.1; noribogaine: *m/z* 297.4→122.1, 159.1 and 160.1; clonazepam-d_4: *m/z* 319.9→218.1, 245.1 and 274.1). Ibogaine and noribogaine were identified in all autopsy samples. Hair segmentation was not possible as hair was very short and frizzy. Concentrations of 1.2 and 2.5 ng/mg, respectively were identified. Neither other drugs (licit or illicit) nor alcohol were found. The presence of ibogaine and noribogaine in all autopsy samples was consistent with the recent consumption of *Tabernanthe iboga* and this was assumed to be responsible of the drowning fatality. The history of exposure, regarding hair analysis, is dis-

cussed. LC-MS/MS appears to be the best method for analyzing complex and poorly volatile alkaloids in autopsy samples and particularly in hair due to the presence of a nitrogen ring and the relatively low concentrations to be measured

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Int J Legal Med 2008 **122** (2) 109
LC-MS assay for quantitative determination of cardio glycoside in human blood samples
LC-MS analysis of the cardio glycosides digoxin and digitoxin in biological samples is described. The method was optimized for use in the forensic field and was employed for the determination in whole blood and tissue samples. Sample cleanup by solid phase extraction (SPE) on a functionalized polymeric phase resulted in a limit to matrix suppression of <10% for all analytes. Chromatographic separation was achieved using an RP-8 column. Detection of the cardio glycosides was performed with EI in the positive mode and the system was run in single ion monitoring mode. Sodium adducts $(M + Na)^+$ of the analyte and of the internal standard were monitored respectively. The method was fully validated in the analysis of blood samples and successfully applied to forensic cases. The method was accurate and precise over a linear concentration range up to 50 ng/g blood. Lower limit of quantitation was 0.2 ng/g for digoxin and 2 ng/g for digitoxin, respectively. A deuterated analyte was used as the internal standard and so a new microwave-enhanced method for the fast preparation of the labelled analyte within 20 min is presented

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Electrophoresis 2008 **29** (2) 526-537
Separation of very hydrophobic analytes by micellar electrokinetic chromatography. I. Optimization of the composition of the sample solution for the determination of the aromatic ingredients of sassafras and other essential oils of forensic interest
A micellar electrokinetic chromatographic method using UV and (UV)LIF detection in-line was developed for the determination of aromatic constituents, mainly allylbenzenes in essential oils. The method optimization included the enhancement of the composition of the separation electrolyte using ACN and urea to reduce retention factors and $CaCl_2$ to widen the migration time window. Moreover, it was necessary to optimize the composition of the sample solution and this included the addition of a neutral surfactant at high concentration. With the optimal method, the determination of minor constituents in essential oils was possible despite of the presence of a structurally similar compound in a molar ratio excess of 1000:1. The use of UV and LIF-detection in-line allowed the direct comparison of the two detection traces using an electrophoretic mobility x-axis instead of the normal time-based scale. This facilitates the assignment of signals and enhances repeatability. The method developed was successfully applied to the determination of minor and major constituents in herbal essential oils, some of which are forensically relevant as sources of precursors for synthetic drugs

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Forensic Sci Int 2008 **179** (1) 78
Dominance of pre-analytical over analytical variation for measurement of methadone and its main metabolite in postmortem femoral blood
Postmortem blood specimens were simultaneously sampled from the left and right femoral veins and the pre-analytical variation of methadone measurements was evaluated and compared to the analytical variation. A series of 27 duplicate samples from routine autopsy cases comprising mainly drug addicts was employed. A chiral LC-MS/MS method was used for measurement of the *R*- and *S*-enantiomers of methadone and its main metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium (EDDP). The analytical CV% was determined to be in the range 3-4% for methadone enantiomers and 4-6% for EDDP enantiomers. The total measurement uncertainty (CV_T) was estimated from the pre-analytical variation (CV_{PA}), analytical variation proper (CV_A), and variation related to calibration (traceability) (CV_{Cal}) according to the relationship $CV_T = [CV^2_{PA} + CV^2_A + CV^2_{Cal}](0.5)$. Uncertainty related to calibration concerned a component related to the purity of drug reference compound and a contribution from the production of calibrator solutions (CV_{Cal}<1%). Pre-analytical sampling variation was investigated using the duplicate measurements of blood samples after subtraction of the analytical component. The pre-analytical variation amounted to a CV% of 19-21% for *R*- and *S*-methadone and 30-38% for *R*- and *S*-EDDP, i.e. considerably greater than the other components. Due to the squared addition principle, the resulting total uncertainty (CV_T) became largely identical to the CV_{PA}, i.e. 19-21% for *R*- and *S*-methadone and 31-38% for *R*- and *S*-EDDP enantiomers. Accordingly, CV_T exceeded CV_A by a factor 5 or more. Dominance of the pre-analytical component of variation may also be likely for other compounds measured in postmortem blood samples. Therefore, the width of the 95%-uncertainty interval (+/-$2CV_T$) for a postmortem measurement is largely determined by the pre-analytical component of variation. This considered should be taken into account when judging on the uncertainty of postmortem measurement results

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Forensic Sci Int 2008 **179** (2-3) 176
Nails of newborns in monitoring drug exposure during pregnancy
Cocaine, benzoylecgonine, morphine, methadone, caffeine, nicotine, and cotinine were determined in nail samples from the first 3 months of life of 25 newborns abandoned immediately after birth (group 1) and of 33 babies born at the local maternity hospital whose families were recruited on a voluntary basis (group 2) in order to investigate *in utero* drug exposure. All substances were examined by gas chromatography-mass spectrometry (detection limit: 0.025 ng/mg). In addition, results were compared with mothers' self-reported habits when the information was available. In group 1, 12 nails were found positive for caffeine and 13 for both nicotine and cotinine. Six samples tested cocaine- (range, median: 0.14-0.25, 0.175 ng/mg) and benzoylecgonine-positive (range, median: 0.12-0.20, 0.165 ng/mg). Both nicotine and cocaine were always extracted together with their main metabolite. Morphine was found in four samples (range, median: 0.10-0.15, 0.125 ng/mg), methadone in five samples (range, median: 0.12-0.26, 0.170 ng/mg) that were found negative for all other compounds. In group 2, two samples tested positive for methadone (0.16, 0.17 ng/mg). The mothers self-report of the use of coffee always corresponded to caffeine positivity in the newborn nails (n=6), whereas six samples tested positive for nicotine and/or cotinine with a non-smoking mother. Sixteen out of the 33 samples of group 2 proved negative for all compounds. This demonstrates for the first time that once that sample collection problems are solved, nails of the first period of life may be employed as an indicator of in utero drug exposure

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J Anal Toxicol 2008 **32** (2) 147
Quantitation of seven low-dosage antipsychotic drugs in human postmortem blood using LC-MS-MS
Antipsychotic drugs are of considerable interest In forensic toxicology because of their abuse potential and involvement in intoxications and suicides. Recently, several new drugs utilised at low level doses have appeared on the market and thus required further of demands on assays to monitor them. This work is intended to develop a validated liquid chromatography-tandem mass spectrometry assay for the quantitation of the low-dosage antipsychotic drugs buspirone, fluphenazine, flupenthixol, perphenazine, risperidone, ziprasidone, and zuclopenthixol in human postmortem blood. Following liquid-liquid extraction using methyl *t*-butyl ether, compounds were separated on a Zorbax SB-CN column. Calibration curves were linear in the range 0.8-100 µg/L ($r > 0.998$) for all drugs. Both within- and between-day coefficients of variation were lower than 25% for all drugs at the LOQ, and extraction recoveries ranged between 58 and 112%. The possibility of matrix effects were closely investigated. Fifty-four authentic samples were analyzed within the routine postmortem investigation and this resulted in the diagnosis of three fatal intoxications. Whereas only a few intoxications were identified, the assay may present valuable information on suicidal deaths in psychotic patients where a true negative result implies noncompliance and a higher susceptibility for suicide. In the absence of a sensitive enough method, this conclusion is not possible. Therefore, we believe that antipsychotic drugs must be measured not only in toxic concentrations but also in therapeutic levels in postmortem cases

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J Chromatogr B 2008 **869** (1-2) 9
Determination of amobarbital and phenobarbital in serum by gas chromatography-mass spectrometry with addition of formic acid to the solvent
Gas chromatography-mass spectrometry (GC-MS) for the rapid and accurate quantification of amobarbital and phenobarbital has been developed without the use without derivatization. Whereas the compounds measured without derivatization showed low sensitivity because of adsorption, addition of 3% formic acid to the solvent improved the sensitivity for the analytes. With respect to the matrix effect, solid-phase and liquid-liquid extraction from serum were investigated. The correlation coefficients of the calibration curves were 0.9995 or better, and the accuracy and precision of intraday and interday assays were consistent with Food and Drug Administration (FDA) criteria

13 Alcohol

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Int J Legal Med 2008 **122** (3) 245
Detection of ethyl glucuronide in dried human blood using LC-MS/MS
Ethyl glucuronide has proven useful as a long-term marker in many forensic applications as a direct metabolite of ethanol metabolism. The inability to determine ethyl glucuronide in dried blood was an omission in many investigations. Here, a new technique is described based on mass spectrometry in a Pauli-type ion trap in order to determine this substance in dried blood samples

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Forensic Sci Int 2008 **179** (2-3) 192

Determination of serum alcohol using a disposable biosensor

A disposable biosensor to detect serum alcohol concentration is described. The biosensor was fabricated using the cross-linking method to immobilize alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide (NAD^+) on a screen-printed electrode modified with Meldola's Blue (MB) absorbed on Nafion. It employs the electrocatalytic properties of MB as an electron transfer mediator, which catalyzes the oxidation of NADH to NAD^+ at a low oxidizing potential, thus avoiding interferences due to the presence of oxidizable substances in the actual serum samples. The biosensor response for alcohol was investigated in terms of pH, buffer solution, temperature and some interferents. It provides good specificity, reproducibility, stability, accuracy and provides a fast response. The biosensor has been satisfactorily employed for the measurement of serum alcohol

14 Tobacco

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J Chromatogr B 2008 **863** (1) 107

Quantification of nicotine, cotinine, *trans*-3'-hydroxycotinine, nornicotine and norcotinine in human meconium by liquid chromatography/tandem mass spectrometry

No analytical methods exist that quantify nicotine, cotinine, *trans*-3'-hydroxycotinine, nornicotine and norcotinine simultaneously in human meconium. Such a technique could improve identification of in utero tobacco exposure, determine if maternal dose-meconium concentration relationships exist, and whether nicotine meconium concentrations predict neonatal outcomes. The first liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry technique for simultaneous quantification of these analytes in meconium was developed and validated. Specimen preparation employed homogenization, enzyme hydrolysis and solid phase extraction. The linear range was 1.25 or 5-500ng/g. Technique applicability was evaluated with meconium collected from an *in utero* tobacco exposed infant

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J Chromatogr B 2008 **865** (1-2) 13

Enantiomeric analysis of anatabine, nornicotine and anabasine in commercial tobacco by multi-dimensional gas chromatography and mass spectrometry

Analysis of the enantiomeric compositions of anatabine, nornicotine and anabasine in commercial tobacco by development of a fully automated multi-dimensional gas chromatography (MDGC) system with a megabore precolumn and cyclodextrin-based analytical column is described. The enantiomer abundances of anatabine and nornicotine varied among different tobacco. *S*-(-)-anatabine, as a proportion of total anatabine, was 86.6% for flue-cured, 86.0% for burley and 77.5% for oriental tobacco. *S*-(-)-nornicotine, as a proportion of total nornicotine, was 90.8% in oriental tobacco and higher than in burley (69.4%) and flue-cured (58.7%) tobacco. *S*-(-)-anabasine, as a proportion of total anabasine, was relatively constant for flue-cured (60.1%), burley (65.1%) and oriental (61.7%) tobacco. A simple solvent extraction with dichloromethane followed by derivatisation with trifluoroacetic anhydride resulted in relative standard deviations of less than 1.5% for the determination of the *S*-(-)-isomers of all three alkaloids. The study also suggested that a higher proportion of *S*-(-)-nornicotine is related to the more active nicotine demethylation in the leaf

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Clin Chim Acta 2008 **388** (1-2) 228

Analysis of cotinine in dried blood spots by LC APCI tandem mass spectrometry (Letter)

No abstract available

15 Homeland Security

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J Anal Toxicol 2008 **32** (1) 44

Multianalyte quantification of five sesqui- and ethyl ether oxy-mustard metabolites in human urine by liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry

Sesqui- and oxy-mustards pose a significant threat to military forces and civilians because they are potent vesicants. An isotope-dilution high-performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry method has been developed employing negative ion multiple reaction monitoring for the analysis of sesqui-mustard metabolites bis(2-hydroxyethylthio)alkanes (n = 1-5) and oxy-mustard metabolite bis(2-hydroxyethylthioethyl)ether in human urine. Relative standard deviations were < 10% and the reportable limits of detection were 1 ng/mL in 0.5 mL of urine.The method was applied to 100 samples collected from individuals with no known exposure to sesqui- or oxy-mustards, and no urines showed detectable levels of any of the analytes,indicating that these metabolites may be used for monitoring exposure to sesqui- and oxy-mustards

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Forensic Sci Int 2008 **179** (2-3) 98

Forensic analysis of bioagents by X-ray and TOF-SIMS hyperspectral imaging

Hyperspectral imaging combined with multivariate statistics is an approach to microanalysis that makes the full use of the large amount of data potentially collected in forensics analysis. This investigation examines the efficacy of using hyperspectral imaging-enabled microscopies to identify chemical signatures in simulated bioagent materials. This technique facilitated the ready discrimination between all samples in the test. In particular, the hyperspectral imaging approach allowed for the identification of particles with trace elements that would have been missed with a more traditional approach to forensic microanalysis. The importance of combining signals from multiple length scales and analytical sensitivities is discussed

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J Anal Toxicol 2008 **32** (1) 57

Gas chromatography-tandem mass spectrometry analysis of red blood cells from Gottingen minipig following whole-body vapor exposure to VX

A technique is described to detect fluoride ion generated *O*-ethyl methylphosphonofluoridate (VX-G) in Göttingen minipig red blood cells (RBC) following whole-body exposure to VX vapor utilizing a gas chromatograph-tandem mass spectrometer (GC-MS-MS). Dose-response curves for VX exposure were produced following application of the fluoride ion reactivation assay to the RBC fraction of serially collected whole blood samples that were taken after whole-body exposures that varied in both duration and concentration. GC-MS-MS analysis of minipig RBC samples after 180-min exposures at two different concentrations was a more precise indicator for severity of exposure than the assay of acetylcholinesterase (AChE) inhibition for the same samples. AChE enzyme activity recovered faster than indicated by the apparent elimination rate of VX-G. GC-MS-MS analyses of RBC samples following VX exposure demonstrate this method has both adequate sensitivity and specificity to indicate the severity of exposure

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J Anal Toxicol 2008 **32** (1) 37

Gas chromatographic-mass spectrometric analysis of sulfur mustard-plasma protein adducts: Validation and use in a rat inhalation model

Sulfur mustard (HD) is an alkylating agent that quickly reacts with macromolecular targets and this results in the formation of stable adducts producing depots for markers of exposure. The purpose of this study was to validate an analytical procedure for detection of HD-plasma protein adducts and to confirm the utility of the method in an HD rat inhalation study. Calibration curves were prepared in human and rat plasma at six levels of HD (12.5 to 400 nM). Correlation coefficients for the mean data were 0.9987 for human and 0.9992 for rat plasma. The percent coefficient of variation (%CV) derived from the mean concentration data ranged from 0.53 to 14.1% in human (n = 5) and 0.57 to 10.63% in rat (n = 6) plasma. Intraday and interday precision and accuracy studies were carried out at three concentration levels (25, 150, 300 nM) to represent low, medium, and high concentrations of HD relative to those employed in the calibration curve. Precision and accuracy were measured by determining %CV and % error, respectively. For intra- and interday studies, the %CVs and absolute % errors were less than 15%. The limits of quantitation for human and and rat plasma were 20.88 nM and 16.73 nM respectively. In animal studies, rats were exposed to nebulized HD at six doses. The data indicate a dose-dependent relationship between maximal plasma concentrations and dose administered (R^2 = 0.9728). Results from this study reflect an accurate, precise, and sensitive method. The method was useful in determining plasma protein adduct formation in a rat inhalation model

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J Chromatogr B 2008 **870** (1) 91

Development of an automated on-line pepsin digestion-liquid chromatography-tandem mass spectrometry configuration for the rapid analysis of protein adducts of chemical warfare agents

Key issues in protection against and non-proliferation of chemical warfare agents (CWA) are rapid monitoring and retrospective verification. These may be adequately accomplished by the analysis of persistent protein adducts of these agents. Liquid chromatography-mass spectrometry (LC-MS) is the technique of choice in the analysis of such protein adducts but the overall experimental procedure is quite complicated. Therefore, an automated on-line pepsin digestion-LC-MS configuration has been produced for the rapid determination of CWA protein adducts. The utility of this configuration is demonstrated by the analysis of specific adducts of sarin and sulfur mustard to human butyryl cholinesterase and human serum albumin, respectively

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J Anal Toxicol 2008 **32** (1) 78

Quantification of sarin and cyclosarin metabolites isopropyl methylphosphonic acid and cyclohexyl methylphosphonic acid in minipig plasma isotope-dilution and liquid chromatography-time of flight mass spectrometry

An analytical technique for determining isopropyl methylphosphonic acid (IMPA) and cyclohexyl methylphosphonic acid (CMPA), the metabolic hydrolysis products of toxic organophosphorus nerve agents isopropyl methylphosphonofluoridate (sarin, GB) and cyclohexyl methylphosphonofluoridate (cyclosarin, GF), respectively, has been developed and validated using high-performance liquid chromatography-mass spectrometry with negative ion electrospray ionization with time-of-flight detection (LC-ESI-MS-TOF). The linear range of quantitation was 5 to 125 ng/mL in plasma with a method detection limit of 2 ng/mL for each compound. This method was produced to determine the amount of metabolic hydrolysis that was formed during and after nerve agent exposure in minipigs to account for a major pathway of GB and GF elimination that had not been previously characterized in the bloodstream, particularly during low-level whole-body inhalation experiments. Metabolic hydrolysis accounted for 70% to 90% of the recoverable agent in the bloodstream following exposure when compared to both unbound and cholinesterase bound agent recovered by fluoride ion reactivation analysis for the same samples. The estimated half-life of IMPA and CMPA in plasma was determined to be 44 and 61 min, respectively. The technique employs the mass selectivity of LC-ESI-MS-TOF using a bench-top instrument to accomplish a detection limit that is consistent with reported LC-MS-MS methods analyzing blood samples

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J Chromatogr A 2008 **1202** (2) 124

Detection of chemical warfare agent degradation products in foods using liquid chromatography coupled to inductively coupled plasma mass spectrometry and electrospray ionization mass spectrometry

A technique for the analysis of chemical warfare agent degradation products (CWADPs) is described employing three chromatographic separations in combination with inductively coupled plasma mass spectrometry (ICP-MS). The robust ionization of ICP is virtually matrix independent so enabling the investigation of sample matrices normally considered too complicated for analysis by electrospray ionization (ESI) or atmospheric pressure chemical ionization MS where there is little to no sample preparation. The analysis was focused on detecting CWADPs in food matrices because they present possible vehicles for terrorist contamination. Specific detection of ^{31}P by ICP-MS made resolution of analytes of interest from other P-containing interferences (H_3PO_4) a crucial part of each separation. Up to 10 CWADPs were isolated in the presence of H_3PO_4 with detection limits in the low part per billion levels. Furthermore, one method was tailored to be compatible with both ICP-MS and ESI-MS making structural verification possible

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J Chromatogr A 2008 **1178** (1-2) 239

Field-amplified sample stacking for the detection of chemical warfare agent degradation products in low-conductivity matrices by capillary electrophoresis-mass spectrometry

Preconcentration of chemical warfare agent degradation products (alkylphosphonic acids and alkyl alkylphosphonic acids) in low-conductivity matrices (purified water, tap water and local river water) by field-amplified sample stacking (FASS) was performed by capillary electrophoresis (CE) coupled to ion trap mass spectrometry. FASS was carried out by adding a mixture of $HCOONH_4$ and NH_4OH to the sample which allowed control of the conductivity and the pH in order to obtain performances independent of analyte concentration. The influence of different parameters on FASS (sample to background electrolyte (BGE) conductivity ratio, injection volume and concentration of BGE) were optimized. A good correlation was noted between the bulk electroosmotic velocity predicted by this model and the experimental value deduced from the migration time of the electroosmotic flow marker detected by mass spectrometry (MS). This newly developed method was successfully used in the analysis of tap water and local river water fortified with the analytes. A 10-fold sensitivity enhancement in comparison to the signal obtained without preconcentration procedure resulted. Peak area reproducability obtained in the 0.5-5 μg mL^{-1} concentration range allowed quantitative. Limits of detection of 0.25-0.5 μg mL^{-1} for the alkyl alkylphosphonic acids and of 0.35-5 μg mL^{-1} for the alkylphosphonic acids were obtained in tap water and river water

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J Anal Toxicol 2008 **32** (1) 31

Improvements in the methodology of monitoring sulfur mustard exposure by gas chromatography-mass spectrometry analysis of cleaved and derivatized blood protein adducts

An analytical method for determining exposure to 2,2'-dichlorodiethyl sulfide (sulfur mustard, HD) has been improved. The method is based on the cleavage of adducted HD (protein-hydroxyethylthioethyl esters) to produce thiodiglycol. Following cleavage, a deuterated internal standard was added, and the analytes extracted, derivatized, and analyzed by gas chromatography-negative ion chemical ionization-mass spectrometry. Addition of a concentration step, incorporation of solid sodium bicarbonate to neutralize excess derivatization reagent, and optimization of method and instrument conditions provided dramatic increases in signal-to-noise ratio. A five-day precision and accuracy study was conducted, including interday and intraday unknown analysis. Linearity was verified by a $R^2 > 0.9995$ for all five curves evaluated. The precision and accuracy of the assay were demonstrated to be excellent by analysis of the interday and intraday unknown samples (< 10% relative standard deviation and relative error in most cases). Statistical treatment of the method blanks and calibration results demonstrated a lowering of the limit of quantitation from 25 nM (HD, human plasma, *in vitro*) to 1.56 nM. Sample and calibration stability through the analytical sequence was established by the inclusion of continuing calibration verification standards (< 5% error). Short-term sample stability was confirmed by reinjection of a calibration set after 18 days ($R^2 = 0.9997$). Quantitative agreement with the previous method was supported by the analysis of a 50 nM standard protein sample (HD, rat plasma) with both methodologies (< 1% error)

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J Anal Toxicol 2008 **32** (1) 63

A rapid and sensitive technique for assessing exposure to VX *via* GC-MS-MS analysis

A rapid and sensitive technique for the analysis of the chemical warfare agent VX in plasma taken from Gottingen minipigs has been produced using isotope-dilution gas chromatography-tandem mass spectrometry (GC-MS-MS). Chromatographic separation was achieved on a 5% diphenyl/95% dimethyl polysiloxane capillary column with a total run time of about 11 min. The analyte was detected using ammonia chemical ionization in the multiple reaction monitoring mode after a simple isolation with 10% 2-propanol in hexane. A good linear relationship was obtained in the quantitative concentration range of 10 ng/mL to 1000 ng/mL ($r^2 = 0.9998$) with an average slope of 1.275 +/- 0.037 (n = 7), and an absolute detection limit of 0.4 pg on column. The average recovery for VX was 95% in saline in the concentration range of 50-100 ng/mL. The technique was successfully employed in the analysis of VX in minipig plasma in a preliminary toxicokinetic study

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J Anal Toxicol 2008 **32** (1) 73

Determination of VX-G analogue in red blood cells *via* gas chromatography-tandem mass spectrometry following an accidental exposure to VX

A sensitive technique to identify exposure to the chemical warfare agent VX is described in which the biomarker ethyl methylphosphonofluoridate (VX-G) is measured in red blood cells (RBCs) after treatment with fluoride ion using isotope-dilution gas chromatography-tandem mass spectrometry. The analyte was extracted *via* solid-phase extraction and detected employing ammonia chemical ionization in the multiple reaction monitoring mode. A good linear relationship was obtained in the quantitative concentration range of 4 ng/mL to 1000 ng/mL with an absolute detection limit of < 1 pg on column. The technique has been employed in the analysis of RBCs from a laboratory worker accidentally exposed to VX vapor. As late as 27 days following exposure, detection and quantitation of VX-G was still possible

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J Anal Toxicol 2008 **32** (1) 92

Validation and application of a gas chromatography-mass spectrometry method for determining soman concentration in rat plasma following low-level vapor exposure

A technique for the determination of the chemical warfare agent soman (GD) in rat plasma has been validated and applied to low-level inhalation exposure studies currently being conducted. The technique employs a fluoride ion-based regeneration assay with isotope dilution followed by large volume injection gas chromatography with ammonia chemical ionization mass spectrometric detection. After sample preparation by solid phase extraction, chro-

matographic isolation was achieved using a 14% cyanopropylphenyl/86% dimethyl polysiloxane capillary column with a total run time of 18.16 min. Soman and the deuterated isotope ($^2H_4$-soman) internal standard were ascertained with the selected ion monitoring mode and quantitated using the ammonia adduction ratio of *m/z* ions 200/204. A reproducible linear relationship was produced for the quantitative concentration range of 10 pg on-column to 1000 pg on-column (r^2 = 0.9995) for standards in ethyl acetate with a detection limit of 5.65 pg on-column, and an average recovery of 93% in plasma. This sensitive technique was successfully employed in the analysis of soman in rat plasma shortly post-exposure, resulting in the construction of dose-response plots

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J Anal Toxicol 2008 **32** (1) 86

A gas chromatographic-mass spectrometric approach to examining stereoselective interaction of human serum proteins with soman

The organophosphorus (OP) nerve agent soman (GD) contains two chiral centers (a carbon and a phosphorus atom), resulting in four stereoisomers (C+P+, C-P+, C+P-, and C-P-); the P- isomers exhibit a mammalian toxicity that is approximately 1000-fold greater than that of the P+ isomers. The ability to measure the binding or hydrolysis of each of the four stereoisomers is important in the development of enzymes with the potential to protect against GD intoxication. Employing a gas chromatography-mass spectrometry-based approach, the capacity of plasma-derived human serum albumin, plasma-purified human butyrylcholinesterase, goat milk-derived recombinant human butyrylcholinesterase, and recombinant human paraoxonase 1 to interact with each of the four stereoisomers of GD *in vitro* at pH 7.4 and 25°C was measured. In these experimental conditions, the butyrylcholinesterase samples were found to bind GD with a relative preference for the more toxic stereoisomers (C-P- > C+P- > C-P+ > C+P+), while human serum albumin and paraoxonase 1 interacted with GD with a relative preference for the less toxic isomers (C-P+/C+P+ > C+P-/C-P-). The results suggest that these human proteins exhibit distinct stereoselective interactions with GD. The developed technique may be employed to rapidly assess substrate stereospecificity, enhancing future efforts to develop more effective organophosphorus bioscavenger proteins

16 Workplace

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J Chromatogr A 2008 **1190** (1-2) 333

Comparative evaluation of liquid chromatography-mass spectrometry *versus* gas chromatography-mass spectrometry for the determination of hexabromocyclododecanes and their degradation products in indoor dust

Gas chromatography-electron-capture negative ionization-mass spectrometry (GC-ECNI/MS) and liquid chromatography-electrospray tandem mass spectrometry (LC-ESI/MS/MS) was employed to analyse domestic and office dust samples (*n*=37) for hexabromocyclododecanes (HBCDs). To optimize the method to quantify HBCDs using GC-ECNI/MS, BDE 128 was used as internal standard (I.S.) in all samples, while ^{13}C-labeled α-HBCD was used as I.S. in some samples. Total HBCD concentrations (sum of α-, β-, and γ-HBCD diastereomers) were calculated using response factors (RFs) for α- and γ-HBCD as individual diastereomers and employing an average RF for both diastereomers. Statistical comparison showed that concentrations obtained *via* GC-ECNI/MS were statistically indistinguishable (p0.05) from those obtained using LC-ESI/MS/MS. The closest match between the two techniques was obtained using [^{13}C]α-HBCD as I.S. and the average RF for α- and γ-HBCDs. Excellent linear correlations (Pearson coefficient values r>0.9) resulted between the GC-ECNI/MS and LC-ESI/MS/MS results, with slopes ranging from 0.76 to 1.36. Pentabromocyclododecenes (four isomers) and tetrabromocyclododecadienes (two isomers) were detected in the studied samples and were identified as degradation products of HBCDs after separation from the parent compound on the basis of both retention time and mass spectrum. This finding indicate that the elimination of HBr is the major degradation pathway for HBCDs in dust

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Rapid Commun Mass Spectrom 2008 **22** (4) 471

Identification of 2,3-dimethyl-2,3-diisobutyl succinonitrile in laser printer emissions

2,3-Dimethyl-2,3-diisobutyl succinonitrile was identified as the main volatile organic compound (>90%) emitted from laser printers during the printing process. Experiments were carried out in a large environmental chamber of 30 m^3, where the printers were placed and working simulating 'real office setting' conditions. Air samples were taken on Tenax TA adsorbent cartridges in the vicinity of the printers and further analyzed by thermal desorption gas chromatography/mass spectrometry (TDGC/MS). The structure of the compound has been determined and is presented in this study. Additional data obtained by nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) spectroscopy, and liquid chromatography/tandem mass spectrometry (LC/MS/MS) support the proposed structure, with no reported CAS number, as 2,3-dimethyl-2,3-diisobutyl succinonitrile. It is a byproduct of the thermal decomposition of 2,2'-azobis(2,4-dimethyl valeronitrile), a commercially available free radical polymerization initiator used in polymerization processes during the manufacture of the toners. By means of head-space GC/MS, 15 toners used in black & white and colour printers have been investigated. Six of them contained 2,3-dimethyl-2,3-diisobutyl succinonitrile, which has also been detected in the respective processed paper

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J Chromatogr B 2008 **870** (1) 98

Miniaturized hollow fiber assisted liquid-phase microextraction with *in situ* derivatization and gas chromatography-mass spectrometry for analysis of bisphenol A in human urine sample

A new technique is described for the determination of trace amounts of bisphenol A (BPA) in human urine samples. This involves miniaturized hollow fiber assisted liquid-phase microextraction (HF-LPME) with in situ derivatization and gas chromatography-mass spectrometry (GC-MS). The detection limit and the quantification limit of BPA in human urine sample are 0.02 and 0.1 ng ml^{-1} (ppb), respectively. The calibration curve for BPA is linear with a correlation coefficient of >0.999 in the range of 0.1-50 ng ml^{-1}. The average recoveries of BPA in human urine samples spiked with 1 and 5 ng ml^{-1} BPA are 101.0 (R.S.D.: 6.7%) and 98.8 (R.S.D.: 1.8%), respectively, with correction using the added surrogate standard, bisphenol A-$^{13}C_{12}$. This simple, accurate, sensitive and selective analytical technique may be applicable to the determination of trace amounts of bisphenol A in human urine samples

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Rapid Commun Mass Spectrom 2008 **22** (2) 143

Analysis of phenoxyacetic acid herbicides as biomarkers in human urine using liquid chromatography/triple quadrupole mass spectrometry

Phenoxyacetic acids are widely used herbicides. The toxicity of phenoxyacetic acids is debated, but high-level exposure has been shown to be hepatotoxic as well as nephrotoxic in animal studies. An inter-species difference in toxic effects has been found, with dogs particularly susceptible. In this study a method using liquid chromatography/triple quadrupole mass spectrometry (LC/MS/MS) is described for the analysis of 4-chloro-2-methylphenoxyacetic acid (MCPA), and its metabolite 4-chloro-2-hydroxymethylphenoxyacetic acid (HMCPA), 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in human urine. The urine samples were treated by acid hydrolysis to degrade possible conjugations. The sample preparation was performed using solid-phase extraction. Analysis was carried out using selected reaction monitoring (SRM) in the negative ion mode. Quantification of the phenoxyacetic acids was performed using [$^2H_3$]-labeled MCPA and 2,4-D as internal standards. The method was linear in the range 0.05-310 ng/mL urine and has a within-run precision of 2-5%. The between-run precision in lower concentration ranges was between 6-15% and between 2-8% in higher concentration ranges. The limit of detection was determined to 0.05 ng/mL. The metabolites in urine were found to be stable during storage at -20°C. To validate the phenoxyacetic acids as biomarkers of exposure, the method was applied in a human experimental oral exposure to MCPA, 2,4-D and 2,4,5-T. Two healthy volunteers received 200 µg of each phenoxyacetic acid in a single oral dose followed by urine sampling for 72 h post-exposure. After exposure, between 90 and 101% of the dose was recovered in the urine. In the female subject, 23%, and in the male subject 17%, of MCPA was excreted as HMCPA

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J Chromatogr Sci 2008 **46** (1) 53

Isolation and recovery of selected polybrominated diphenyl ethers from human serum and sheep serum: Coupling reversed-phase disk extraction and liquid-liquid extraction techniques with a capillary gas chromatographic electron capture negative ion mass spectrometric determinative technique

PBDEs were isolated from human serum *via* liquid-liquid extraction and column chromatographic cleanup and fractionation with quantitation using capillary GC-MS with electron capture negative ion and selected ion monitoring. They were found in unspiked serum. An alternative sample preparation approach was developed using sheep serum that utilizes a formic acid pre-treatment followed by reversed-phase solid-phase disk extraction and normal-phase solid-phase cleanup using acidified silica gel that yields >50% recoveries. When the recoveries were combined with a minimized phase ratio for human serum and very low instrument detection limits, levels below 500 ppt were obtained

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Anal Bioanal Chem 2008 **392** (1-2) 97
Modelling of adsorption kinetics and calibration curves of gaseous volatile organic compounds with adsorptive solid-phase microextraction fibre: Toluene and acetone for indoor air applications
Volatile organic compounds (VOCs) at trace levels in air were investigatin using solid-phase microextraction (SPME) with adsorptive Carboxen/PDMS fibre. However, owing to competitive adsorption, quantification remains a challenging task. A theoretical model, based on Fick's laws and an extended Langmuir equation, is suggested to account for the adsorption kinetics of acetone/toluene mixture on SPME fibre under various static extraction conditions. The semipredictive model was first used to determine the axial diffusion coefficients of analytes in the sampling device. The model was then tested with a complex VOC mixture and demonstrated good agreement with experimental data

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J Chromatogr B 2008 **862** (1-2) 93
Quantitative determination of paraquat in meconium by sodium borohydride-nickel chloride chemical reduction and gas chromatography/mass spectrometry (GC/MS)
The aim of this study was to produce a technique for the GC/MS assay of paraquat in meconium as a biomarker of fetal exposure to paraquat. The technique employed a sodium borohydride-nickel chloride reduction procedure, liquid-liquid extraction of the perhydrogenated product, concentration, and GC/MS assay. The technique showed good overall recovery (102.56%) with %CV (inter-assay) of less than 13%, and a limit of detection of 0.0156μg/g. Analysis of meconium samples taken from a study population in the Philippines (*n*=70) demonstrated a 2.8% prevalence of fetal exposure to to the herbicide paraquat

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J Chromatogr B 2008 **863** (1) 115
Validation of an HPLC-MS/MS method for the simultaneous determination of phenylmercapturic acid, benzylmercapturic acid and *o*-methylbenzyl mercapturic acid in urine as biomarkers of exposure to benzene, toluene and xylenes
A liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique was produced and fully validated for the simultaneous determination of phenylmercapturic acid, benzylmercapturic acid and *o*-methylbenzyl mercapturic acid in human urine as biomarkers of exposure to benzene, toluene and xylenes (BTX) according to U.S. Food and Drug Administration guidance. After solid phase extraction and LC separation, samples were analyzed by a triple-quadrupole mass spectrometer employing negative ion mode, using isotope-labeled analogs as internal standards (ISs). The technique is adequate for all the validation criteria required. The limits of detection of the three analytes, ranging from 0.30 to 0.40μgl^{-1}, and the high throughput make the technique suitable for the routine biological monitoring of co-exposure to BTX both in the occupational and environmental settings. The validated technique was employed to assess exposure to BTX in a group of 354 urban traffic wardens

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J Chromatogr B 2008 **867** (1) 26
Validation of an HPLC/MS/MS method with isotopic dilution for quantitative determination of *trans,trans*-muconic acid in urine samples of workers exposed to low benzene concentrations
Urinary *trans,trans*-muconic acid (*t,t*-MA), a biomarker of benzene exposure, is usually determined by HPLC methods with detection by either UV or, more recently, electrospray tandem mass spectrometry. However, not all these methods have been fully validated for quantitative analysis. Herein is presented an HPLC/MS/MS method for reliable quantitative determination of *t,t*-MA that employs a commercial deuterium-labeled isotope as internal standard; the matrix effect has been evaluated and LOD is 0.22 μg/L. This method was utilised to analyse 200 urine samples, 175 of them collected at end-of-shift from workers in an oil refinery

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J Chromatogr A 2008 **1190** (1-2) 286
Improved accuracy in the determination of polycyclic aromatic hydrocarbons in air using 24 h sampling on a mixed bed followed by thermal desorption capillary gas chromatography-mass spectrometry
A new analytical technique for the determination of polycyclic aromatic hydrocarbons (PAHs) in ambient air has been developed. The technique employs active sampling on sorption tubes consisting of polydimethylsiloxane (PDMS) foam, PDMS particles and a TENAX TA bed. After sampling, the solutes are quantitatively recovered by thermal desorption and analysed by capillary GC-MS. The new sampling technique was compared to the classical method using high-volume sampling on a glass fiber filter followed by polyurethane foam for 24h sampling of ambient air. Volumes enriched were 144 l on the mixed bed and 1296 m^3 with the classical method. The concentrations measured using the new method were significantly higher that the values obtained using the classical method, i.e. a factor 1.2-3 for the high molecular weight PAHs and up to 35 times for naphthalene and 23 times for acenaphthene. The total toxicity equivalence value (TEQ) for PAHs was around two times higher compared to the conventional method, suggesting that the concentrations of PAHs in ambient air have been underestimated until now. Notable figures (mean value for 17 PAHs) of the method are repeatability 7.4%, detection limit 13 pg/m^3, accuracy 105.6% and linearity 0.996. The technique also provides interesting perspectives for the analysis of other semi-volatile persistent organic pollutants (POPs) in air as illustrated by profiling of polychlorinated biphenyls (PCBs) at a workplace during removal of transformer oil

17 Product Authenticity

Choi DM, Park S, Yoon TH, Jeong HK, Pyo JS, Park J, Kim D, Kwon SW*// *Korea Food & Drug Admin, 194 Tongilro, Eunpyeong gu, Seoul 122 704, South Korea
J AOAC Int 2008 **91** (3) 580
Determination of analogs of sildenafil and vardenafil in foods by column liquid chromatography with a photodiode array detector, mass spectrometry, and nuclear magnetic resonance spectrometry
Two analogs of sildenafil and vardenafil were detected by column liquid chromatography (LC) with a photodiode array detector in food. They were isolated by preparative LC; their structures were provided by mass spectrometry and nuclear magnetic resonance spectrometry. One analog was identified as methisosildenafil (compound A), 5-(5-(3,5-dimethylpiperazin-1-ylsulfonyl)-2-ethoxy-phenyl)-1-methyl-3-propyl-1*H*-pyrazolo[4,3-*d*]-pyrimidin-7(6*H*)-one. This is a sildenafil analog with a dimethylpiperazine ring substituted for the methylpiperazine group. The second analog, hydroxyvardenafil (compound B) is reported for the first time in this study. The International Union of Pure and Applied Chemistry name for hydroxyvardenafil is 2-(2-ethoxy-5-(4-(2-hydroxyethyl)-piperazin-1-ylsulfonyl)phenyl)-5-methyl-7-propyl-imidazo[1,5-*f*][1,2,4]triazin-4(3*H*)-one. The novel vardenafil analog has a hydroxyl group added to the ethylpiperazine group

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Anal Chim Acta 2008 **623** (2) 178
Assessment of hand-held Raman instrumentation for *in situ* screening for potentially counterfeit artesunate antimalarial tablets by FT-Raman spectroscopy and direct ionization mass spectrometry
Pharmaceutical counterfeiting has become a significant public health problem worldwide and new, rapid, user-friendly, reliable and inexpensive techniques for drug quality screening are required. Herein is described an illustration of the chemical characterization of genuine and fake artesunate antimalarial tablets by portable Raman spectroscopy and validation by FT-Raman spectroscopy and ambient mass spectrometry. The practicality of a compact and robust portable Raman spectrometer (TruScan) for the in situ chemical identification of counterfeit tablets is reported

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Anal Biochem 2008 **379** (2) 182
Detection of protein modifications and countefeit protein pharmaceuticals using isotope tags for relative and absolute quantification and matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry: Studies of insulins
A method is described for the detection of modifications to active pharmaceutical ingredients derived from recombinant DNA technologies and to detect counterfeit drug products. Isotope tags for relative and absolute quantification (iTRAQ) reagent coupled with matrix-assisted laser desorption/ionization tandem time-of-flight (MALDI-TOF/TOF) mass spectrometry was evaluated as both a qualitative and quantitative analytical method. Five types of insulin (human, bovine, porcine, Lispro, and Lantus) were employed as examples because of their minor variations in amino acid sequence. Several experiments were conducted in which each insulin variant was separately digested with Glu-C, and the products were labeled with one of four different iTRAQ reagents. Subsequently all products were combined for desalting and MALDI-TOF/TOF mass spectrometric analysis. Following optimization, the digestion procedure resulted in insulin sequence coverage of 100%. Five different types of insulin were readily identified, including human insulin (P28K29) and Lispro insulin (K28P29), which differ only by the interchange of two adjacent residues. Furthermore, quantitative analyses show that the results obtained from the iTRAQ method are consistent with those determined by other conventional methods. Collectively, the iTRAQ method may be used as a qualitative and quantitative technique for the detection of protein modification and counterfeiting

18 Techniques

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J Chromatogr B 2008 **870** (1) 32

Application of dried blood spots combined with HPLC-MS/MS for the quantitfication of acetaminophen in toxicokinetic studies

Quantitative bioanalysis of acetaminophen in dried blood spots (DBS) was achieved with a reversed phase HPLC-MS/MS technique. The method was developed and validated employing small volumes (15 µL) of dog blood. Samples were extracted for analysis with methanol. Detection was by positive ion TurboIonSpray ionisation combined with selected reaction monitoring MS. The analytical concentration range was 0.1-50 µg/mL. The intra-day precision and bias values were both less than 15%. Acetaminophen was stable in DBS stored at room temperature for at least 10 days. The technique was applied in a toxicokinetic (TK) study where the data obtained from DBS samples was physiologically comparable with results from duplicate blood samples (diluted 1:1 (v/v) with water) analysed using identical HPLC-MS/MS conditions. This work illustrates that quantitative analysis of a drug extracted from DBS may provide high quality TK data while minimising the volume of blood withdrawn from experimental animals, by an order of magnitude lower than is current practice in the pharmaceutical industry. This is the first reported application of DBS analysis to a TK study in support of a safety assessment study. The success of this and similar, related studies has resulted in the aim to apply DBS technology as the recommended analytical approach for the assessment of pharmacokinetics (PK)/TK for all new oral small molecule drug candidates which have previously demonstrated a successful bioanalytical validation

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J Therm Anal Calorim 2008 **93** (2) 553

Thermal stability evaluation of doping compounds before GC-MS analysis by DSC

The use of anabolic androgenic steroids, ß-agonists, stimulant and narcotic compounds to improve athletic performance is banned by the Medical Commission of the International Olympic Committee. Herein is evaluated the thermal stability of 17 compounds by the use of the DSC for their potential GC-MS analysis either under free form or under TMS derivative form. In DSC, esterified and unesterified anabolic steroids were analyzed by a true melting peak, followed by a large exothermic peak at about 251–316°C due to oxidative degradation. Analysis was by GC-MS mainly under TMS derivatives. Hydroxylated and unhydroxylated stimulant compounds (xanthines) appeared to be more stable at high temperature. As unhydroxylated xanthines were not silylated with BSTFA - TMCS, their GC analysis was carried out as their free forms. TMS derivatisation of albuterol hemisulfate and codeine phosphate is preferable. In the present study, analysis by GC-MS of all 17 doping compounds in the same GC-MS run, the optimal silylation temperature and best column initial temperature were determined at both 60°C

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J Sep Sci 2008 **31** (2) 402

Analysis of volatile components of drugs and explosives by solid phase microextraction-ion mobility spectrometry

Current ion mobility spectrometry (IMS) devices were used to detect drugs and explosives in the form of particles and, in cases where the vapor pressure of the drugs or explosives was sufficiently high, the gas was sampled and detected directly. The objective of this study was to demonstrate the use of solid phase microextraction (SPME) as a preconcentration technique coupled to an IMS for the detection of odor signature compounds of drugs and explosives. The reduced mobilities (K_o) and IMS operating conditions for the odor signature compounds of cocaine, marijuana, and 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) are reported for the first time. LODs, linear dynamic ranges (LDRs), and the precision of the analysis of these odor signature compounds, and the explosive taggant 2,3-dimethyl-2,3-dinitrobutane (DMNB) were obtained by SPME-IMS and normal IMS conditions. The systematic optimization of the IMS operating parameters for the detection of these odor compounds is also reported utilizing genetic algorithms (GAs) to locate the optimal settings for the detection of these compounds of interest. These results support the case for targeting volatile components as a presumptive detection for the presence of the parent compounds of drugs and explosives. Furthermore, the IMS-specific GA developed may be used as an optimization tool for the detection of other compounds of interest in future studies

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Forensic Sci Int 2008 **174** (2-3) 259

Feasibility of source identification of seized street drug samples by exploiting differences in isotopic composition at natural abundance level by GC/MS as compared to isotope ratio mass spectrometry (IRMS) (Letter)

No abstract available

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J Anal Toxicol 2008 **32** (3) 220

Development and validation of ELISA and GC-MS procedures for the quantification of dextromethorphan and its main metabolite dextrophan in urine and oral fluid

The detection of dextromethorphan and its major metabolite dextrorphan in urine and oral fluid following the development of a highly sensitive enzyme-linked immunosorbent assay and gas chromatography-mass spectrometry confirmation method is described. For the screening assay, the intraday precision was less than 8% for urine and less than 5% for oral fluid. The interday precision was less than 10% for both drugs in urine and oral fluid. For the confirmatory procedure, both inter- and intraday precision was less than 5% for both matrices. The detection limit for both methods was 1 ng/mL. The quantifying ions chosen from the full scan mass spectra were *m/z* 271 for dextromethorphan, *m/z* 329 for dextrorphan, and *m/z* 332 for tri-deuterated dextrorphan-d_3. A high recovery yield (> 93%) from the Quantisal oral fluid collection device resulted and the drugs were stable in the collection device for at least 10 days at room temperature. The extracted drugs from both matrices were stable for at least 48 h while kept at room temperature. Both screening and confirmatory techniques were applied to genuine urine and oral fluid specimens obtained from volunteers following therapeutic ingestion of dextromethorphan

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J Am Soc Mass Spectrom 2008 **19** (1) 151

Gas phase reaction of substituted isoquinolines to carboxylic acids in ion trap and triple quadrupole mass spectrometers after electrospray ionization and collision-induced dissociation

Bisubstituted isoquinolines that possess great potential as prolylhydroxylase inhibitor drug candidates (e.g., FG-2216). Unusually favored gas-phase formations of carboxylic acids after collisional activation were observed during MS. The protonated molecule of [(1-chloro-4-hydroxy-isoquinoline-3-carbonyl)-amino]-acetic acid was dissociated, yielding the 1-chloro-4-hydroxy-isoquinoline-3-carboxylic acid methyleneamide cation. Further dissociation resulted in the nominal elimination of 11 u that was the product of the loss of HCN and simultaneous addition of oxygen to the product ion and this formed the protonated 1-chloro-4-hydroxy-isoquinoline-3-carboxylic acid. The preference of this structure under mass spectrometric conditions was verified by tandem mass spectrometry analyses using the corresponding methyl ester (1-chloro-4-hydroxy-isoquinoline-3-carboxylic acid methyl ester) that eliminated methylene (-14 u) upon collisional activation. Furthermore, the major production of 1-chloro-4-hydroxy-isoquinoline-3-carboxylic acid, which was the product of the loss of water in MS^3 experiments, restored the precursor ion structure by re-addition of H_2O. Confirmation of these phenomena was obtained by chemical synthesis of proposed gas-phase intermediates, H/D exchange experiments, high-resolution/high accuracy mass spectrometry at MS^n level, and "ping-pong" analyses (MS^7, in which the precursor ion was dissociated and the respective product ion isolated to regenerate the precursor ion for repeated dissociation. On the basis of these results, dissociation pathways for [(1-chloro-4-hydroxy-isoquinoline-3-carbonyl)-amino]-acetic acid were proposed that may be further utilized for the characterization of structurally related compounds or metabolic products in clinical, forensic, or doping control analysis

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Anal Chim Acta 2008 **621** (2) 185

Carrier-mediated liquid phase microextraction coupled with high performance liquid chromatography for determination of illicit drugs in human urine

A new technique coupling carrier-mediated liquid phase microextraction (LPME) to high performance liquid chromatography (HPLC) is described for the analysis of illicit drugs in human urine. Following an appropriate carrier in organic phase, simultaneous extraction and enrichment of hydrophilic (morphine and ephedrine) and hydrophobic (pethidine) drugs were achieved. Effects of the types of organic solvents and carriers, the carrier concentration in the organic phase, the HCl concentration in the acceptor solution, the stirring rate, and the extraction time on the enrichment factor of analytes were examined. Under the optimal experimental conditions, high enrichment factors (202-515) were obtained. The linear detection ranges were 0.1-10mgL^{-1} for the studied drugs. The limits of detection (LOD) at signal-to-noise ratio of 3 were 0.05mgL^{-1} for both morphine and ephedrine, and 0.02mgL^{-1} for pethidine. This technique was successfully applied to analysis of ephedrine in actual urine specimens demonstrating that the determination of illicit drugs in urine was feasible