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Current awareness in drug testing and analysis

1 Reviews

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J Chromatogr B 2010 **878** (17-18) 1391
Tropane alkaloid analysis by chromatographic and electrophoretic techniques: An update
Atropine is an example of a class of compounds termed tropane alkaloids which may be employed as antidotes to mitigate organophosphorus intoxication. However, atropine has a toxicity and so requires closely monitoring during treatment. Therefore, simple, fast, and sensitive methods of analysis for tropane alkaloids in serum are necessary. Analysis is usually achieved by such methods as gas chromatography (GC); high performance liquid chromatography (HPLC), and capillary electrophoresis (CE). In HPLC, specialised columns augment resolution efficacy. A variety of liquid and solid capillary fillings used in micellar electrokinetic chromatography, microemulsion electrokinetic chromatography, capillary electrochromatography, and enantioseparation confer high versatility to CE. Ultraviolet light detection was frequently employed. However, now sensitivity and analyte identification have been enhanced by coupling GC, HPLC, and CE to mass spectrometry. In addition medical deployment, tropane alkaloids, and in particular cocaine, are abused. Forensic analysis of tropane alkaloids and their metabolites involves the additional difficulty of incontrovertible drug identification. Severe legal consequences are a hazard. Therefore, sophisticated analytical methods have been developed and may provide additional approaches to therapeutic drug monitoring. This review includes examples from forensic cocaine analysis and from doping analysis

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Alcohol Clin Exp Res 2010 **34** (6) 941
Advancing alcohol biomarkers research
Biomarkers to detect past alcohol use and identify alcohol-related diseases have long been pursued as important tools for research into alcohol use disorders as well as for clinical and treatment applications and other settings. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) sponsored a workshop titled "Workshop on Biomarkers for Alcohol-Induced Disorders" in June 2008. The intent of this workshop was to review and discuss recent progress in the development and implementation of biomarkers for alcohol use and alcohol-related disorders with a goal to formulate a set of recommendations to use to stimulate and advance research progress in this critical area of alcoholism research. Presentations at this workshop reviewed the current status of alcohol biomarkers, providing a summary of the history of biomarkers and the major goals of alcohol biomarker research. Moreover, presentations provided a comprehensive overview of the current status of several well-recognized biomarkers of alcohol use, a summary of recent studies to characterize novel biomarkers and their validation, along with perspectives and experiences from other NIH institutes and from other federal agencies and industry, related to regulatory issues. Following these presentations, a panel discussion focused on a set of issues presented by the organizers of this workshop. These discussion points addressed: (i) issues related to strategies to be adopted to stimulate biomarker discovery and application, (ii) the relevance of animal studies in biomarker development and the status of biomarkers in basic science studies, and (iii) issues related to the opportunities for clinical and commercial applications. This article summarizes these perspectives and highlights topics that constituted the basis for recommendations to enhance alcohol biomarker research

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J Chromatogr B 2010 **878** (17-18) 1207
History and perspectives of bioanalytical methods for chemical warfare agent detection
The development of bioanalytical methods for chemical warfare (CW) agents and their biological markers of exposure is reviewed. Emphasis is placed on a more detailed assessment of techniques for organophosphorus nerve agents. Bioanalytical approaches for unaltered CW agents are employed particularly for toxicokinetic/toxicodynamic analyses. Nerve agents differ in biological activity and detoxification pathways of enantiomers. CW agents exist in human body for a relatively short period. They are hydrolysed, metabolised, or adducted to nucleophilic sites on macromolecules such as proteins and DNA. Such compounds may be investigated as biological markers of exposure. Over the past 20 years, metabolites, protein adducts of nerve agents, vesicants and phosgene, and DNA adducts of sulfur and nitrogen mustards, have been identified and characterized. Detection has been enabled by the development of sensitive analytical techniques based primaily on mass spectrometry in combination with gas or liquid chromatography. Biomarkers for sarin, VX and sulfur mustard have been confirmed in instances of accidental and deliberate human exposures.

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J Anal Toxicol 2010 **34** (4) 169
Postmortem aviation forensic toxicology: An overview
The subtopic aviation combustion toxicology in the field of aerospace toxicology has already been overviewed previously. This overview continues the theme with a summary of the findings associated with postmortem aviation forensic toxicology. The literature between 1960-2007 was searched and salient findings related to postmortem toxicology evaluated. Following a brief introduction, this overview is divided into the sections of analytical methods; carboxyhemoglobin and blood cyanide ion; ethanol; drugs; result interpretation; glucose and hemoglobin A_{1c}; and references. This overview should provide an outline source for aviation forensic toxicology within the field of aerospace toxicology

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.

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Alcohol Clin Exp Res 2010 **34** (6) 946
Future prospects for biomarkers of alcohol consumption and alcohol-induced disorders
The lack of reliable measures of alcohol intake is a major obstacle to the diagnosis, treatment, and research of alcohol abuse and alcoholism. Successful development of a biomarker that allows for accurate assessment of alcohol intake and drinking patterns would not only be a major advance in clinical care but also a valuable research tool. A number of advances have been made in testing the validity of proposed biomarkers as well as in identifying potential new biomarkers through systems biology approaches. This commentary will examine the definition of a biomarker of heavy drinking, the types of potential biomarkers, the steps in biomarker development, the current state of biomarker development, and critical obstacles for the field. The challenges in developing biomarkers for alcohol treatment and research are similar to those found in other fields. However, the alcohol research field must reach a competitive level of rigor and organization. It is recommended that NIAAA consider taking a leadership role in organizing investigators in the field and providing a common set of clinical specimens for biomarker validation studies

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Alcohol Clin Exp Res 2010 **34** (6) 968
Clinical (nonforensic) application of ethyl glucuronide measurement: Are we ready?
Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are minor metabolites of ethanol. Multiple studies have documented that, depending upon the amount of alcohol consumed, they can be measured in biological fluids for hours to days after the parent compound can no longer be detected. Testing for the presence of EtG, in a manner analogous to urinary drug abuse screening, has largely been restricted to forensic and law enforcement situations. Despite a real need for an objective and possibly quantitative marker of ethanol exposure for use in conjunction with outpatient clinical trials and treatment programs, measurement of these metabolites has seen only limited clinical application. The barriers to more extensive clinical use of EtG/EtS testing, particularly misleading assay results that can occur as a consequence of inadvertent exposure to nonbeverage ethanol-containing substances, are reviewed and put into perspective. Additional information needed to develop guidelines for optimal clinical utilization of EtG/EtS measurements is discussed

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J Chromatogr B 2010 **878** (17-18) 1365
Use of NMR techniques for toxic organophosphorus compound profiling
Selected examples to demonstrate the versatility of nuclear magnetic resonance (NMR) spectroscopy in the analysis of toxic organophosphorus (OP) compounds [OP pesticides and chemical warfare agents (CWAs)] are reviewed. Applications of biological importance, for example, studies on inhibition mechanism, metabolism, and exposure determination are presented. In addition, the environmental analysis of OP compounds by NMR spectroscopy is reviewed. Environmental and food sample residue analysis are discussed. Also, the characterization of degradation products in the environment. Some NMR in support of the Chemical Weapons Convention (e.g. the development of suitable CWA detoxification means) and technique development for verification analysis for CWAs and their degradation products are discussed

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Alcohol Clin Exp Res 2010 **34** (6) 955
Alcohol biomarkers in applied settings: Recent advances and future research opportunities
During the past decade, advances have been made in the identification, development, and application of alcohol biomarkers. This is important because of the unique functions that alcohol biomarkers can serve in various applied settings. To carry out these functions, biomarkers must display several features including validity, reliability, adequacy of temporal window of assessment, reasonable cost, and transportability. During the past two decades, several traditional alcohol biomarkers have been studied in multiple human studies. Meanwhile, several new, promising biomarkers, including various alcohol metabolites and alcohol biosensors, are being explored in human studies. In addition, researchers have explored using biomarkers in combination and using biomarkers in combination with self-reports, resulting in increased sensitivity with little sacrifice in specificity. Despite these advances, more research is needed to validate biomarkers, especially the new ones, in humans. Moreover, recent advances in high-throughput technologies for genomics, proteomics, and metabolomics offer unique opportunities to discover novel biomarkers, while additional research is needed to perfect newly developed alcohol sensors. Development of more accurate biomarkers will help practicing clinicians to more effectively screen and monitor individuals who suffer from alcohol use disorders

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Ther Drug Monit 2010 **32** (3) 324
Perspectives of liquid chromatography coupled to low- and high-resolution mass spectrometry for screening, identification and quantification of drugs in clinical and forensic toxicology
The use of liquid chromatography coupled to low- and high-resolution mass spectrometry in clinical and forensic toxicology is reviewed. Included is targetted and for more comprehensive screening of drugs (of abuse), identification of drug metabolites, and multianalyte procedures for quantification of drugs and/or their metabolites in body samples. In addition to a critical overview, perspectives are discussed

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Anal Bioanal Chem 2010 **397** (3) 1039
Analytical methods for abused drugs in hair and their applications
Hair has received attention for its applicability as an alternative matrix to blood and urine for determining drugs of abuse in fields such as forensic and toxicological sciences. It is non-invasive and may be employed to elucidate a prolonged intake of drugs of abuse (along the length of the hair) compared with blood and urine. Several pretreatment steps are necessary. These include washing out contaminants, extraction of target compounds and cleanup for instrumental analysis. Each procedure necessitates characteristic and independent features for the class of drugs, e.g., stimulants, narcotics, cannabis, and other medicaments. Recently developed approaches to determine drugs of abuse are reviewed and summarized. Pretreatment steps, sensitivity and applicability are critically discussed

2 Sports Doping - General

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J Chromatogr A 2010 **1217** (25) 4109
Fast analysis of doping agents in urine by ultra-high-pressure liquid chromatography-quadrupole time-of-flight mass spectrometry. II: Confirmatory analysis
In terms of doping control, samples are usually analysed in two steps. Firstly, a rapid screening test and in the case of a positive result, secondly, a confirmatory analysis. In order to screen and confirm 103 doping agents from various classes (e.g., β-blockers, stimulants, diuretics, and narcotics) a two-step approach based on ultra-high-pressure liquid chromatography coupled to a quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) was developed. The screening approach was described in a previous paper (i.e., Fast analysis of doping agents in urine by ultra-high-pressure liquid chromatography-quadrupole time-of-flight mass spectrometry. Part I: screening analysis). For confirmatory analysis, basic, neutral and acidic compounds were extracted by a dedicated solid-phase extraction (SPE) in a 96-well plate format. They were then detected by MS in the tandem mode to obtain precursor and characteristic product ions. The mass accuracy and the elemental composition of precursor and product ions were employed for compound identification. The approach was validated including matrix effect determination. Subsequently the technique was deemed sufficiently accurate to confirm suspect results unambiguously according to the positivity criteria established by the World Anti-Doping Agency (WADA). Furthermore, an isocratic method was developed to separate ephedrine from its isomer pseudoephedrine and cathine from phenylpropanolamine in a single run. This facilitated their direct urinalysis

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Eur J Mass Spectrom 2010 **16** (3) 301
Mass spectrometry-based characterization of new drugs and methods of performance manipulation in doping control analysis
Frequent updating and proactive, preventive anti-doping research is essential for efficient and comprehensive sports drug testing. A vital aspect is the incorporation of novel, emerging drugs into routine doping controls. Several new drugs and drug candidates with abuse potential were investigated. These included the so-called Rycals (ryanodine receptor calstabin complex stabilizers, e.g. S-107), hypoxia-inducible factor (HIF) stabilizers, and peroxisome-proliferator-activated receptor (PPAR) δ agonists (e.g. GW1516). Studies employed different mass spectrometry- and ion mobility-based approaches. Gas phase dissociation behaviors of the drugs were elucidated. Detailed knowledge of fragmentation routes expedites more rapid identification of metabolites. In addition, it facilitates analysis of structurally related, presumably "tailor-made", analogs with potential use doping. The applicability of product ion characterization was illustrated with GW1516. Oxidation products were easily identified in urine samples using diagnostic fragment ions as measured with high resolution/high accuracy mass spectrometry and higher energy collision-induced dissociation (HCD)

3 Steroids

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Rapid Commun Mass Spectrom 2010 **24** (13) 1881

Determination of nandrolone metabolites in human urine: Comparison between liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectrometry

Nandrolone (19-nortestosterone) is an androgenic anabolic steroid illegally used as a growth-promoting agent in animal breeding and as a performance enhancer in athletics. Therefore, its use was officially banned in 1974 by the Medical Commission of the International Olympic Committee (IOC). Following nandrolone administration, the main metabolites in humans are 19-norandrosterone, 19-norethiocolanolone and 19-norepiandrosterone, and their presence in urine is the basis of detecting its abuse. The present work was undertaken to determine, in human urine, nandrolone metabolites (phase I and phase II) by developing and comparing multiresidue liquid chromatography/tandem mass spectrometry (LC/MS/MS) and gas chromatography/mass spectrometry (GC/MS) methods. A double extraction by solid-phase extraction (SPE) was necessary for the complete elimination of the interfering compounds. The proposed methods were also tested on a real positive sample, and they allow us to determine the conjugated/free fractions ratio reducing the risk of false positive or misleading results and they should allow laboratories involved in doping control analysis to monitor the illegal use of steroids. The advantages of LC/MS/MS over GC/MS (which is the technique mainly used) include the elimination of the hydrolysis and derivatization steps: it is known that during enzymatic hydrolysis several steroids can be converted into related compounds and deconjugation is not always 100% effective. The validation parameters for the two methods were similar (limit of quantification (LOQ) <1 ng/ml and percentage coefficient of variance (CV%) <16.4), and both were able to confirm unambiguously all the analytes, thus confirming the validity of both techniques

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Rapid Commun Mass Spectrom 2010 **24** (16) 2375

Improved ultrasonic-based sample treatment for the screening of anabolic steroids by gas chromatography/mass spectrometry

A rapid sample treatment procedure for the gas chromatography/mass spectrometry (GC/MS) determination of anabolic steroids in human urine has been developed. The new procedure makes use of ultrasonic energy to reduce reaction times and increase the overall sensitivity. The following variables affecting the performance of the ultrasonic treatment were optimised: (i) time, (ii) device, (iii) frequency, and (iv) temperature. It was found that, under an ultrasonic field, the hydrolysis of conjugated steroids with β-glucuronidase from *Escherichia coli* K12 was possible with a treatment time of 10 min. The accuracy and precision of the ultrasonic method were found to be in agreement with those achieved with the conventional thermal conductivity procedure (Student's t-test; $p = 0.05$, $n = 10$). After the enzymatic hydrolysis, the derivatisation of the target compounds with trimethylsilyl (TMS) reagent, methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA)/NH_4I/dithioerythritol (DTE) (1000:2:4, v/w/w), was also accelerated using ultrasonic energy. In order to test the applicability of the use of ultrasonic energy in the acceleration of the derivatisation reaction with TMS, the classic method of thermal conductivity was applied for comparative purposes to a pool of 35 androgenic anabolic steroids (AAS) and/or their metabolites. The results demonstrated that after 3 min of sonication in a Sonoreactor device (50% amplitude), 19 of the 35 compounds studied showed similar reaction yield to those obtained with the classic procedure requiring 30 min (Student's t-test; $p = 0.05$, $n = 5$); 13 increased to higher silylation yields; and for the steroids 1-testosterone, danazol and etiocholanolone-D_5, the same results were obtained using a sonication time of 5 min. The overall applicability of the ultrasonic-based sample treatment method is shown by the analysis of five urine samples. The results are similar to those achieved by the routine procedure. The new method is fast, robust, and allows high sample throughput

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J Pharm Biomed Anal 2010 **52** (5) 727

Determination of anabolic steroids in human urine by automated in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry

The analysis of seven anabolic steroids (boldenone, nandrolone, testosterone, methyltestosterone, epiandrosterone, androsterone, and atnozolol) in human urine has been accomplished by the development of a simple, rapid and sensitive procedure. Firstly, β-glucuronidase was employed to hydrolyze the glucuronide-conjugates. Subsequently, on-line in-tube solid-phase microextraction (SPME) coupled with liquid chromatography-mass spectrometry (LC-MS) was employed for the analysis. The steroids were separated within 14 min by high performance liquid chromatography utilising a Chromolith RP-18e column and 5 mM ammonium formate/methanol (35/65, v/v) as a mobile phase at a flow rate of 1.0 ml/min. Electrospray ionization parameters in the positive ion mode were optimized for the MS analysis. The optimum in-tube SPME conditions were 20 draw/eject cycles with a sample size of 40 µl using a Supel-Q PLOT capillary column for extraction. Extracted analytes could be desorbed readily from the capillary column by flow of the mobile phase. No carryover was noted. Using the in-tube SPME LC-MS with SIM mode detection anhd with the exception for stanozolol, good linearity of the calibration curve ($r > 0.995$) was produced in the concentration range of 0.5-20 ng/ml. Detection limits (S/N = 3) of anabolic steroids were in the range 9-182 pg/ml and the developed procedure demonstrated a 20-33-fold greater sensitivity than the direct injection method. Within-day and between-day precisions were below 4.0% and 7.3% ($n = 5$), respectively. Urine samples were successfully analyzed without interference peaks. Urine samples siked with anabolic steroids showed recovery rates above 85%. The described approach should faciliatate urine analysis for anti-doping tests

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Rapid Commun Mass Spectrom 2010 **24** (15) 2245

Mass spectrometric characterization of urinary metabolites of the selective androgen receptor modulator andarine (S-4) for routine doping control purposes

Selective androgen receptor modulators (SARMs) are potent anabolic agents with tissue-selective properties. Due to their potential misuse in elite sport, the World Anti-Doping Agency (WADA) has prohibited SARMs since 2008, and although no representative drug candidate has yet received full clinical approval, recent findings of SARMs illegally sold *via* the internet have further supported the need to efficiently test for these compounds in doping controls. In the present communication, the mass spectrometric characterization of urinary metabolites of the SARM Andarine (also referred to as S-4) compared with earlier *in vitr*o and animal studies is reported. Liquid chromatography interfaced to high-resolution/high-accuracy (tandem) mass spectrometry was used to identify phase I and II metabolites, confirming the predicted target analytes for sports drug testing purposes including the glucuronic acid conjugates of the active drug, its monohydroxylated and/or deacetylated product, the hydrolysis product resulting from the removal of the compound's B-ring, as well as the sulfate of the monohydroxylated and the deacetylated phase I metabolite. The obtained data will support future efforts to effectively screen for and confirm the misuse of the non-approved drug candidate Andarine in routine doping control

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Spectrosc Eur 2010 **22** (4) 6

Targeting new performance enhancing drugs in doping controls: Selective androgen receptor modulators (SARMS)

Abstract not available

4 Peptides

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Rapid Commun Mass Spectrom 2010 **24** (14) 2046

Determination of growth hormone secretagogue pralmorelin (GHRP-2) and its metabolite in human urine by liquid chromatography/electrospray ionization tandem mass spectrometry

GHRP-2 (pralmorelin, D-Ala-D-(β-naphthyl)-Ala-Ala-Trp-D-Phe-Lys-NH_2), which belongs to a class of growth hormone secretagogue (GHS), is intravenously used to diagnose growth hormone (GH) deficiency. Because it may be misused in expectation of a growth-promoting effect by athletes, the illicit use of GHS by athletes has been prohibited by the World Anti-Doping Agency (WADA). Therefore, the mass spectrometric identification of urinary GHRP-2 and its metabolite D-Ala-D-(β-naphthyl)-Ala-Ala-OH (AA-3) was studied using liquid chromatography/electrospray ionization tandem mass spectrometry for doping control purposes. The method consists of solid-phase extraction using stable-isotope-labeled GHRP-2 as an internal standard and subsequent ultra-performance liquid chromatography/tandem mass spectrometry, and the two target peptides were determined at urinary concentrations of 0.5-10 ng/ml. The recoveries ranged from 84 to 101%, and the assay precisions were calculated as 1.6-3.8% (intra-day) and 1.9-4.3% (inter-day). Intravenous administration of GHRP-2 in ten male volunteers was studied to demonstrate the applicability of the method. In all ten cases, unchanged GHRP-2 and its specific metabolite AA-3 were detected in urine

6 CNS Agents

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J Anal Toxicol 2010 **34** (2) 73
Confirming urinary excretion of mephentermine and phentermine following the ingestion of oxethazaine by gas chromatography-mass spectrometry analysis
The World Anti-Doping Agency prohibits the use of mephentermine and phentermine in sports. However, they have been detected for the first time in urine specimens following the administration of oxethazaine, a therapeutic medication. In sporting event, a urine specimen donor who tested positive for mephentermine and phentermine. The athlete claimed consumption of Mucaine® for treating stomach pain had produced the positive result. Five volunteers were administrated oxethazaine (a topical anesthetic found in the multi-ingredient medication Mucaine and its generic equivalent, Stoin, both of which are available in Taiwan), mephentermine, and phentermine. Following the administration of these drugs, excretion profiles of mephentermine and phentermine were found to be similar. However, urine specimens collected at different time points following the administration of oxethazine and mephentermine were found to produce characteristically different mephentermine:phentermine ratios

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J Anal Toxicol 2010 **34** (4) 210
Analysis of stimulants in oral fluid and urine by gas chromatography-mass spectrometry II: Pseudoephedrine
Ephedrines are banned to athletes by the World Anti-Doping Agency (WADA), only "in competition" if their concentration in urine exceeds the cut-off limit. An approach for the optimization of the identification and quantification of ephedrines in oral fluid (OF) is described. Following administration of therapeutic doses of pseudoephedrine to various subjects, research was performed to establish if there is a correlation in respect of times of elimination and of concentration trends of ephedrine in OF and urine. Data from urinalysis of eight subjects demonstrated reproducible times of disappearance of ephedrines from OF. At 12 h after administration, pseudoephedrine was generally at low concentrations or undetectable in oral fluid. However, urinalysis for the same period of time showed higher ephedrine concentrations and exceeded cutoff values generally between 8 and 24 h following administration. Within- and between-individual variability was noted in terms of concentrations of pseudoephedrine in OF. Constant pseudoephedrine concentrations in OF were only observed following administration of sustained-release drugs

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Rapid Commun Mass Spectrom 2010 **24** (18) 2706
Toxicological determination and *in vitro* metabolism of the designer drug methylenedioxypyrovalerone (MPDV) by gas chromatography/mass spectrometry and liquid chromatography/quadrupole time-of-flight mass spectrometry
A method for the toxicological screening of the new designer drug methylenedioxypyrovalerone (MDPV) is described; with an emphasis on its application for anti-doping analysis. The metabolism of MDPV was evaluated *in vitro* using human liver microsomes and S9 cellular fractions for CYP450 phase I and uridine 5'-diphosphoglucuronosyltransferase (UGT) and sulfotransferase (SULT) phase II metabolism studies. The resulting metabolites were subsequently liquid/liquid extracted and analyzed using gas chromatography/mass spectrometry (GC/MS) as trimethylsilyl (TMS) derivatives. The structures of the metabolites were further confirmed by accurate mass measurement using a liquid chromatography/quadrupole time-of-flight (LC/QTOF) mass spectrometer. The studies demonstrated that the main metabolites of MDPV are catechol and methyl catechol pyrovalerone, which are in turn sulfated and glucuronated. The method for the determination of MDPV in urine has been fully validated by assessing the limits of detection and quantification, linearity, repeatability, and accuracy. This validation demonstrates the suitability for screening of this stimulant substance for anti-doping and forensic toxicology purposes

7 Equine

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J Chromatogr A 2010 **1217** (28) 4749
The use of a simple backflush technology to improve sample throughput and system robustness in routine gas chromatography tandem mass spectrometry analysis of doping control samples
An approach for the detection of anabolic steroids in equine urine has been developed by means of a simple, low cost system for the backflushing of capillary gas chromatography (GC) columns. This modification was simple to perform and rapid to establish and optimize. Backflushing technology was demonstrated to provide significant improvements in respect of sample throughput and enhanced system robustness

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Biomed Chromatogr 2010 **24** (7) 744
***In vitro* and *in vivo* studies of androst-4-ene-3,6,17-trione in horses by gas chromatography-mass spectrometry**
This paper describes the application of gas chromatography-mass spectrometry (GC-MS) for *in vitro* and *in vivo* studies of 6-OXO in horses, with a special aim to identify the most appropriate target metabolite to be monitored for controlling the administration of 6-OXO in racehorses. *In vitro* studies of 6-OXO were performed using horse liver microsomes. The major biotransformation observed was reduction of one keto group at the C3 or C6 positions. Three *in vitro* metabolites, namely 6α-hydroxyandrost-4-ene-3,17-dione (M1), 3α-hydroxyandrost-4-ene-6,17-dione (M2a) and 3β-hydroxyandrost-4-ene-6,17-dione (M2b) were identified. For the *in vivo* studies, two thoroughbred geldings were each administered orally with 500 mg of androst-4-ene-3,6,17-trione (5 capsules of 6-OXO®) by stomach tubing. The results revealed that 6-OXO was extensively metabolized. The three *in vitro* metabolites (M1, M2a and M2b) identified earlier were all detected in post-administration urine samples. In addition, seven other urinary metabolites, derived from a further reduction of either one of the remaining keto groups or one of the remaining keto groups and the olefin group, were identified. These metabolites included 6α,17β-dihydroxyandrost-4-en-3-one (M3a), 6,17-dihydroxyandrost-4-en-3-one (M3b and M3c), 3β,6β-dihydroxyandrost-4-en-17-one (M4a), 3,6-dihydroxyandrost-4-en-17-one (M4b), 3,6-dihydroxyandrostan-17-one (M5) and 3,17-dihydroxyandrostan-6-one (M6). The longest detection time observed in urine was up to 46 h for the M6 metabolite. For blood samples, the peak 6-OXO plasma concentration was observed 1 h post administration. Plasma 6-OXO decreased rapidly and was not detectable 12 h post administration

8 Recreational Drugs - General

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Electrophoresis 2010 **31** (7) 1256
CEC-ESI ion trap MS of multiple drugs of abuse
This article describes a method for the separation and determination of nine drugs of abuse in human urine, including amphetamines, cocaine, codeine, heroin and morphine. This method was based on SPE on a strong cation exchange cartridge followed by CEC-MS. The CEC experiments were performed in fused silica capillaries (100 µm x 30 cm) packed with a 3 µm cyano derivatized silica stationary phase. A laboratory-made liquid junction interface was used for CEC-MS coupling. The outlet capillary column was connected with an emitter tip that was positioned in front of the MS orifice. A stable electrospray was produced at nanoliter per minute flow rates applying a hydrostatic pressure (few kPa) to the interface. The coupling of packed CEC columns with mass spectrometer as detector, using a liquid junction interface, provided several advantages such as better sensitivity, low dead volume and independent control of the conditions used for CEC separation and ESI analysis. For this purpose, preliminary experiments were carried out in CEC-UV to optimize the proper mobile phase for CEC analysis. Good separation efficiency was achieved for almost all compounds, using a mixture containing ACN and 25 mM ammonium formate buffer at pH 3 (30:70, v/v), as mobile phase and applying a voltage of 12 kV. ESI ion-trap MS detection was performed in the positive ionization mode. A spray liquid, composed by methanol-water (80:20, v/v) and 1% formic acid, was delivered at a nano-flow rate of approximately 200 nl/min. Under optimized CEC-ESI-MS conditions, separation of the investigated drugs was performed within 13 min. CEC-MS and CEC-MS^2 spectra were obtained by providing the unambiguous confirmation of these drugs in urine samples. Method precision was determined with RSDs values <or = 3.3% for retention times and <or = 16.3% for peak areas in both intra-day and day-to-day experiments. LODs were established between 0.78 and 3.12 ng/ml for all compounds. Linearity was satisfactory in the concentration range of interest for all compounds (r^2 >or = 0.995). The developed CEC-MS method was then applied to the analysis of drugs of abuse in spiked urine samples, obtaining recovery data in the range 80-95%

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J Sep Sci 2010 **33** (12) 1767
Gas chromatography-mass spectrometric method for the screening and quantification of illicit drugs and their metabolites in human urine using solid-phase extraction and trimethylsilyl derivatization

A simple and rapid GC-MS method has been developed for the screening and quantification of many illicit drugs and their metabolites in human urine by using automatic SPE and trimethylsilylation. Sixty illicit drugs, including parent drugs and their metabolites that are possibly abused in Korea, can be monitored by this method. Among them, 24 popularly abused illicit drugs were selected for quantification. Very delicate optimizations were carried out in SPE, trimethylsilylation derivatization, and GC/MS to enable such remarkable achievements. Trimethylsilylated analytes were well separated within 21 min by GC-MS. In the validation results, the LOD of all the analytes were in the range of 2-75 ng/ml. The LOQ of the quantified analytes were in the range of 5-98 ng/ml. The linearity (r^2) of the quantified analytes ranged 0.990-1.000 in each concentration range between 10 and 1000 ng/ml. The mean recoveries ranged from 62 to 126% at three different concentrations of each analyte. The inter-day and inter-person accuracies were within - 13.3 approximately 14.9%, and -10.1 approximately 13.0%, respectively, and the inter-day and inter-person precisions were less than 12.9%. The method was reliable and efficient for the screening and quantification of abused illicit drugs in routine urine analysis

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Anal Chem 2010 **82** (13) 5636

Automated online solid phase extraction ultra high performance liquid chromatography method coupled with tandem mass spectrometry for determination of forty-two therapeutic drugs and drugs of abuse in human urine

The simultaneous identification and quantification in human urine of 42 drugs (21 therapeutic and 21 of abuse) with a fast analysis times (around 11 min) has been achieved by development of an automated online solid phase extraction ultra high performance liquid chromatography approach coupled with tandem mass spectrometry (SPE UHPLC-MS/MS). For technique validation, particular attention was accorded to the matrix effect by means of a matrix-matched calibration in blank urine and appropriately diluted. With all the drugs of abuse analysed, the limit of quantitation (LOQ) values were lower than the legal threshold concentration levels. Therefore, the approach was suitable for routine control. The approach was employed for urinalysis of patients positive to the I level screening test

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J Anal Toxicol 2010 **34** (2) 103

Roche DAT immunoassay: Sensitivity and specificity testing for amphetamines, cocaine, and opiates in oral fluid

Testing of oral fluid for drugs of abuse increases apace in the workplace, legal, treatment, and health settings. The recently developed homogeneous Roche DAT screening assays for amphetamines, cocaine metabolite [benzoylecgonine (BZE)], methamphetamines, and opiates in oral fluid were evaluated. Precision and accuracy were assessed using control samples at +/-25% of cutoff. Oral fluid specimens were collected from 994 subjects enrolled in a drug treatment or probation and parole drug-testing program. Sensitivity, specificity and agreement of the Roche DAT assays were compared with liquid chromatography-tandem mass spectrometry (LC-MS-MS). Furthermore, 180 research specimens from Kroll Laboratories were analyzed for amphetamines and methamphetamines. Screening cutoff concentrations were 40 ng/ml for amphetamines, 3 ng/ml for cocaine metabolites, 40 ng/ml for methamphetamines and 10 ng/ml for opiates. LC-MS-MS analyses were performed with cutoff concentrations of 40 ng/ml for amphetamines, 2 ng/ml for BZE, 40 ng/ml for methamphetamines and 10 ng/ml for codeine or morphine. The percent coefficient of variation ranged from 3.4% to 7.3%. Sensitivity and specificity of the Roche DAT assays compared to LC-MS-MS were > 94%, and agreement was > 96% for the four assays. Data indicate that the Roche DAT assays will be an useful alternative to existing more labor-intensive enzyme immunoassays

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J Anal Toxicol 2010 **34** (3) 162

Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin

The combined use of a new designer drug, 4-methylmethcathinone (mephedrone) and heroin resulting in an accidental death is reported. Following the discovery of an unresponsive 22-year-old Caucasian male in his living quarters, he was transported to the hospital where he died. Needle marks were found along the decedent's lower legs and ankles at autopsy and investigators subsequently discovered that the deceased and his roommate had been using "Black Tar" heroin and mephedrone. Toxicological analysis revealed morphine in the deceased's blood at 0.06 mg/l. Furthermore, urinalysis demonstrated 6-acetylmorphine, morphine, codeine, and doxylamine. Liquid-liquid extraction followed by pentafluropropionic anhydride derivatization was employed to isolate mephedrone from both blood and urine specimens. Derivatized extracts were subjected to gas chromatography-mass spectrometry (GC-MS) operating in full-scan mode. For quantitative analysis of mephedrone, GC-MS was operated in selective ion monitoring mode with methamphetamine-d_{14} as an internal standard. Mephedrone was verified in the deceased's blood and urine at 0.50 and 198 mg/l, respectively. To date, the physiological, pharmacological and toxicological effects of mephedrone have not been reported. However, the structural similarities with methcathinone and the high concentration in the decedent's blood resulted in an assumption that the overall contribution of mephedrone to the death was entirely feasible. Consequently, the death was reported as multiple-drug toxicity and the manner as accidental

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J Chromatogr A 2010 **1217** (26) 4402

Qualitative drug analysis of hair extracts by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry

The qualitative analysis of three extracts from hair suspected of containing various psychoactive drugs was performed with an approach utilising comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry (GCxGC/TOFMS). Quantitative analysis was also performed for codeine, morphine, 6-monoacetylmorphine (6-MAM), amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethylamphetamine (MDMA), methadone, and benzylpiperazine (BZP) by liquid chromatography-tandem mass spectrometry (LC-MS/MS). GCxGC/TOFMS provided a non-specific procedure that identified various drugs, metabolites, and impurities not included in the target analysis, for example, cocaine, diazepam, and methaqualone (quaalude). Comprehensive GCxGC separation was accomplished with twin-stage cryo-modulation to focus the eluant from a DB-5 ms (5% phenyl) onto a BPX50 (50% phenyl) GC column. The TOF mass spectrometer provided unit mass resolution in the mass range *m/z* 5-1000 and rapid spectral acquisition (</= 500 spectra/s). Mass spectral deconvolution softwarewas employed to produce clean mass spectra of the individual components. 'Unidentified' components were distinguished following comparison with mass spectra stored in a library database

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Arch Pathol Lab Med 2010 **134** (5) 735

Interpretation and utility of drug abuse immunoassays lessons from laboratory drug testing surveys

Physicians from pain services, drug treatment programs, and the emergency departments often request urinalysis for drugs of abuse in order to aid with patient diagnosis and management. Unfortunately, immunoassays performed on urine have limitations. Therefore, an analysis has been performed to determine and summarize the characteristics, performance, and limitations of urinalysis by immunoassay for drugs of abuse on data collected from the College of American Pathologists Proficiency Testing Surveys. An anlysis was performed on 6 years of urine drug testing proficiency tests. Lysergic acid diethylamide and methaqualone are seldom prescribed or abused Therefore, testing for these substances may be unnecessary. However, implementation of more specific analyses for methylenedioxymethamphetamine and oxycodone may be necessary. It was noted that each drug of abuse immunoassay exhibits a different cross-reactivity profile. Patients with clinically insignificant levels of drugs may have false-positive results depending upon the cross-reactivity profile. Conversely, patients with clinically significant amounts of drugs may have false-negative results. Therefore, laboratory directors should be aware of the characteristics of immunoassays employed by their staff. Any limitations should be communicated to physicians so that qualitative results may be interpreted with the necessary caution. In addition, manufacturer's claims should be thoroughly evaluated and wherever possible in each organization's patient population

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J Anal Toxicol 2010 **34** (3) 155

Detection of ^{9}-tetrahydrocannabinol and amphetamine-type stimulants in oral fluid using the Rapid Stat point-of-collection drug-testing device

The Rapid Stat assay is a point-of-collection drug-testing device for detection of amphetamines, cannabinoids, cocaine, opiates, methadone, and benzodiazepines in oral fluid. It has been evaluated for cannabis and amphetamine-type stimulants. Police officers employed Rapid Stat tests (n = 134) in routine traffic checks. Gas chromatography-mass spectrometry (GC-MS) was employed to analyze oral fluid and blood samples for Δ^9-tetrahydrocannabinol, amphetamine, methamphetamine, methylenedioxymethamphetamine, methylenedioxyethylamphetamine, and methylenedioxyamphetamine. A comparison of GC-MS analysis of oral fluid with the Rapid Stat results for cannabis demonstrated a sensitivity of 85%, a specificity of 87%, and a total confirmation rate of 87%. When compared with serum, the sensitivity of the cannabis assay decreased to 71%, the specificity to 60%, and the total confirmation rate to 66%. These findings may have resulted from an incorrect reading of the THC test results. For oral fluid, comparison of the Rapid Stat amphetamine assay with GC-MS demonstrated a sensitivity of 94%, a specificity of 97%, and a total confirmation rate of 97%. Compared with serum, a sensitivity of 100%, a specificity of 90%, and a total confirmation rate of 92% was noted. Consequently, the amphetamine assay should be regarded as satisfactory

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Forensic Sci Int 2010 **198** (1-3) 11
First nationwide study on driving under the influence of drugs in Switzerland
In Switzerland on 1 January 2005, a two-tier system based on impairment by any psychoactive substances which affect the capacity to drive safely and zero tolerance for certain illicit drugs was enabled. In respect of the new legislation, an offender is confirmed if Δ^9-tetrahydrocannabinol THC is >or= 1.5 ng/ml or amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), cocaine, free morphine are >or= 15 ng/ml in whole blood (confidence interval +/-30%). For all other psychoactive substances, impairment must be demonstrated by applying the so-called "three pillars expertise". In addition, the legal blood alcohol concentration (BAC) limit for driving was reduced from 0.80 to 0.50g/kg. This investigation was performed to measure the prevalence of drugs in the first year after the introduction of the revision of the Swiss Traffic Law in the population of drivers suspected of driving under the influence of drugs (DUID). A database was compiled to collect the data from all DUID cases referred by the police or the Justice to the eight Swiss authorized laboratories between January and December 2005. Data collected were anonymous but included the age, gender, date and time of the event, the type of vehicle, the circumstances, the sampling time and the results of all the performed toxicological analyses. The focus was explicitly on DUID and instances of drivers suspected to be under the influence of alcohol only were not included. There were 4794 DUID offenders comprising 4243 males and 543 females. The mean age of all drivers was 31+/-12 years (range 14-92 years). One or more psychoactive drugs were detected in 89% of all analyzed blood samples and in 11% (n = 530), neither alcohol nor drugs were present. The most frequently encountered drugs in whole blood were cannabinoids (48% of total number of cases), ethanol (35%), cocaine (25%), opiates (10%), amphetamines (7%), benzodiazepines (6%) and methadone (5%). Medicinal drugs such as antidepressants and benzodiazepine-like substances appeared less frequently. Poly-drug use was predominant. However,it may have been underestimated because the laboratories did not always analyze all drugs in a blood sample. This first Swiss study indicates that DUID is a serious problem on the roads in Switzerland. Subsequent studies will illustrate how this situation changes in the following years

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Forensic Sci Int 2010 **198** (1-3) 2
Evaluation of on-site oral fluid screening using Drugwipe-5$^+$, RapidSTAT and Drug Test 5000 for the detection of drugs of abuse in drivers
A major problem worldwide is driving under the influence of drugs. Currently, several countries have adopted a 'per se' legislation to address this problem. A key element to enforcement is the possibility of rapid on-site screening tests to facilitate immediate administrative measures. The reliability of three oral fluid screening devices (Mavand RapidSTAT®, Securetec Drugwipe-5$^+$®, and Drager DrugTest 5000®) have been determined by comparing their on-site results with confirmatory GC-MS plasma analysis. Results demonstrate that the oral fluid on-site devices on the market today are sensitive enough for amphetamine screening. For amphetamine/MDMA screening, RapidSTAT®, Drugwipe-5$^+$®, and DrugTest® 5000 demonstrated respectively sensitivities of 93%, 100% and 92%. For cocaine screening, RapidSTAT®, Drugwipe-5$^+$®, and DrugTest 5000 displayed respectively sensitivities of 75%, 78% and 67%. The studied devices were able to identify about 70% of all cannabis users on site. However, a newer version of the DrugTest 5000® test cassette exhibited a sensitivity of 93%, indicating increased detection of Δ^9-tetrahydrocannabinol using 'new generation' oral fluid screening tests with lowered cut-offs. Following these promising results police officers and judicial experts are enthusiastic to use oral fluid screening devices. They contend that the ease of use and reduced number of false positives when compared with urine screening should result in more roadside tests and more appropriate juridical measures

9 Stimulants

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Chirality 2010 **22** (4) 398
Enantiodiscrimination of methamphetamine by circular dichroism using a porphyrin tweezer
Using exciton-coupled circular dichroism (ECCD) spectroscopy, our lab was able to differentiate between the two enantiomers of methamphetamine using a commercially available porphyrin tweezer as an achiral host. The host-guest complex formed with (+)-(*S*)-methamphetamine produced a negative bisignate-shaped ECCD spectrum, whereas the complex formed with (-)-(*R*)-methamphetamine produced a positive one. This sensitive technique could serve as an alternative method for the enantiodiscrimination of chiral methamphetamine, a commonly abused drug in the United States

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J Sep Sci 2010 **33** (12) 1779
Analysis of cocaine and benzoylecgonine in urine by using multisyringe flow injection analysis-gas chromatography-mass spectrometry system
In this paper, a method was described to determine cocaine (COC) and benzoylecgonine (BZE) in human urine samples by GC-MS detection. The extraction of analytes from urine samples was achieved in an Oasis hydrophilic-lipophilic balance column (20 mm x 3.9 mm id, dp = 25 µm; Waters, USA), incorporated in a multisyringe flow injection system, used for the sample treatment. Finally, to improve the volatility of the BZE, an in-line derivatization reaction with *N*,*O*-bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane was made microwave-assisted in order to reduce the reaction time. The results showed that the proposed method is a good alternative for the analysis of COC and BZE in urine samples because it offers advantages compared with those described in the literature, which include simplicity in the sample treatment, the sensitivity and selectivity necessary to determine the analytes of interest at low levels in the urine and high sample throughput

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J Anal Toxicol 2010 **34** (5) 252
Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in "herbal high" products
The qualitative determination and comparison of individual and relative content of synthetic cannabinoids (cannabinomimetics) in a range of "herbal high" products was determined. By employing mono-isotopic masses derived from the elemental composition of target analytes, liquid chromatography-high resolution accurate mass spectrometry was utilised to rapidly screen samples for a range of cannabinomimetics. A screening database of over 140 compounds was rapidly created. This procedure when combined with further tandem mass spectrometric analyses enabled the detection and identification of compounds for which there were no reference materials. Previously identified cannabinomimetics, such as JWH-018 and CP47,497 and its homologues, were determined in varying relative proportions in addition to several tentatively identified unreported cannabinomimetics. A decision has been made in some countries to include these substances within their drug control legislation. Other countries are considering similar action. However, the currently employed drug screening approaches are not likely to be proficient in providing scientific evidence to support their identification in seized products. The deployment of high-resolution accurate mass spectrometry provides an appropriate method. The approach facilitates a relatively simple and rapid technique for screening products. Substance databases may be constructed. Novel substances may be identified with a combination of accurate mass derived elemental composition and fragment ions combined with fragmentation prediction software

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J Chromatogr A 2010 **1217** (27) 4575
Enantiomeric analysis of drugs of abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry
The enantiomeric analysis of structurally related amphetamines (amphetamine, methamphetamine, 4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA)), ephedrines (ephedrine, pseudoephedrine and norephedrine) and venlafaxine in wastewater has been achieved by the development and validation of an approach employing chiral chromatography coupled with tandem mass spectrometry. Solid-phase extraction employed Oasis HLB sorbent for sample clean-up and concentration of analytes and produced very good recoveries of > 70%. For most studied analytes, signal suppression during MS analysis was negligible. Resolution of enantiomers of chiral drugs was noted to be greater than 1. Preliminary assay validation was undertaken. The mean correlation coefficients of the calibration curves (on average higher than 0.997 for all studied analytes) exhibited good linearity for the approach in the studied range. Intra- and inter-day repeatabilities were on average less than 5%. The technique quantification limits in wastewater were at low ppt levels and varied from 2.25 to 11.75 ng/l. Raw and treated wastewater samples collected from four wastewater treatment plants were analysed. The frequent occurrence of 1*R*,2*S* (-)-ephedrine, 1*S*,2*S* (+)-pseudoephedrine and venlafaxine in both raw and treated wastewater samples was noted. Amphetamine, methamphetamine, MDMA and MDEA were also identified in several wastewater samples. An enantiomeric study of fractions of these chiral drugs demonstrated their variable non-racemic composition. A possible effect of the wastewater treatment processes on the enantiomeric composition of chiral drugs was also observed

and might indicate enantioselective processes occurring during treatment

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J Anal Toxicol 2010 **34** (4) 222

Estimation of the measurement uncertainty by the bottom-up approach for the determination of methamphetamine and amphetamine in urine

By employing a bottom-up approach, the measurement uncertainty (MU) of methamphetamine (MA) and amphetamine (AP) was evaluated in an authentic urine sample with a relatively low concentrations of the drugs. A cause and effect diagram was derived; the amounts of the drugs in the sample, the volume of the sample, method precision, and sample effect were considered uncertainty sources. Urinalysis revealed that the concentrations of the drugs with their expanded uncertainties were 340.5 +/- 33.2 ng/ml for MA and 113.4 +/- 15.4 ng/ml for AP. This indicates that 9.7% (MA) and 13.6% (AP) of the concentration produced an estimated expanded uncertainty. The largest uncertainty was created by sample effect (MA) and technique precision (AP). However, the uncertainty of the volume of the sample was minimal in both. MU should to be assessed during the procedure validation process to estimate test reliability. In addition, the identification of the largest and/or smallest uncertainty source can assist in improving experimental procedures

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Anal Bioanal Chem 2010 **397** (4) 1539

Validation of ELISA screening and LC-MS/MS confirmation methods for cocaine in hair after simple extraction

Hair presents a matrix which is both easily obtainable and may be used to demonstrate drug use over a prolonged period along the length of the shaft. Cocaine and its primary metabolite, benzoylecgonine (BE) in hair have been investigated. A simple, novel extraction procedure has been devised consisting of sonication with H_2O/0.1% formic acid for 4 hours. Extracts was employed both for screening with an enzyme-linked immunoassay (ELISA) and for confirmation by liquid chromatography-tandem mass spectrometry (LC-MS/MS). In accordance with the Society of Hair Testing recommendations, a cutoff of 0.5 ng/mg was employed for the ELISA screening test. LC-MS/MS limits of detection (LODs) were established to be 10 pg/mg for cocaine and 1 pg/mg for BE. Linearity was established over a range of 0.2-5 ng/mg for BE (target analyte) by ELISA. In the LC-MS/MS procedure, the range was 0.10-10 ng/mg for cocaine and 0.01-10 ng/mg for BE. Intra- and interbatch coefficients of variation and mean relative errors were less than 20% for both analytes and all concentrations studied. The validated ELISA and LC-MS/MS procedures were used to analyze 48 hair samples. When the results of both techniques were compared, ELISA demonstrated a sensitivity and specificity of 89.2% and 10.8%

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Anal Bioanal Chem 2010 **397** (3) 1225

-Keto amphetamines: Studies on the metabolism of the designer drug mephedrone and toxicological detection of mephedrone, butylone, and methylone in urine using gas chromatography-mass spectrometry

Recently, a new class of drugs of abuse, for example, mephedrone (bk-4-methylmethamphetamine), butylone (bk-MBDB), and methylone (bk-MDMA), the so-called β-keto (bk) designer drugs has appeared in many countries. It was envisaged that mephedrone, butylone, and methylone would be incorporated within the authors' systematic toxicological analysis (STA) procedure. GC-MS was employed to identify the metabolites of mephedrone in rat and human urine. Six phase I metabolites of mephedrone were detected in rat urine and seven in human urine. These indicated that metabolism followed a pathway of *N*-demethylation to the primary amine, reduction of the keto moiety to the respective alcohol, and oxidation of the tolyl moiety to the corresponding alcohols and carboxylic acid. Rats were treated with doses corresponding to those reported for abuse of amphetamines. Urinalysis by the STA protocol facilitated the detection of mephedrone, butylone, methylone and their metabolites. In addition to macro-based data appraisal, an automated assessment employing the automated mass spectral deconvolution and identification system was conducted. In addition, mephedrone and butylone could be detected in human urine samples submitted for drug testing. If an assumption is made of similar kinetics in humans, the STA protocol applied to urine should be capable of determining proof of an intake of the bk-designer drugs

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J Chromatogr B 2010 **878** (19) 1616

Analysis of amphetamines and metabolites in urine with ultra performance liquid chromatography tandem mass spectrometry

The quantitative determination of seven amphetamines and metabolites in urine was achieved by the development and full validation of a simple, rapid and sensitive ultra performance liquid chromatography tandem mass spectrometry approach. The approach was validated for selectivity, linearity, LOQ, LOD, imprecision, bias, analyte and processed sample stability, matrix effect, recovery, carryover and dilution integrity. A classic liquid-liquid extraction with ethyl acetate was employed as the sample preparation procedure. The compounds were separated on an Acquity UPLC HSS C18 column in 6.8 min. The linear dynamic range was established from 25 to 500 ng/ml. The limit of quantification was set at the lowest calibrator level and the limit of detection ranged from 0.125 to 2.5 ng/ml. The procedure provided excellent intra- and inter-assay imprecision and bias (< 10.7%) at each measured concentration of two external quality controls (QC) and three "in house" QC. No matrix effects were noted and good recoveries (> 70%) were produced for all analytes. No carryover was noted after the analysis of highly concentrated samples (8000 ng/ml). This approach was subsequently applied to authentic samples

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J Anal Toxicol 2010 **34** (2) 57

Urinary excretion of ecgonine and five other cocaine metabolites following controlled oral, intravenous, intranasal, and smoked administration of cocaine

Following controlled administration of oral, intravenous, intranasal, and smoked cocaine, urinary excretion of ecgonine (EC) was compared to that of cocaine, benzoylecgonine, ecgonine methyl ester and minor metabolites, *meta*-hydroxybenzoylecgonine, *para*-hydroxybenzoylecgonine, and norbenzoylecgonine. EC concentrations peaked later than all other analytes but had longer detection times than the other minor metabolites. By emplying 50 ng/ml cutoff concentration and after low doses of 10 to 45 mg cocaine by multiple routes, detection times were extended up to 98 h. Maximum concentrations (C_{max}) of EC were 6-14 mole % of those for benzoylecgonine. C_{max} increased with dose. Time to maximum concentration (T_{max}) was independent of dose. Route of administration did not significantly impact on C_{max} or T_{max} for metabolites. Due to its stability in urine and long detection times, EC is an compound worth considering in order to identify cocaine use

10 Hallucinogens

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Forensic Sci Int 2010 **200** (1-3) 73

GC-MS and GC-IRD analysis of ring and side chain regioisomers of ethoxyphenethylamines related to the controlled substances MDEA, MDMMA and MBDB

Recently, three regioisomeric 3, 4-methylenedioxyphenethylamines with the same molecular weight and major mass spectral fragments of equal mass have been reported as drugs of abuse. These compounds are 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 3,4-methylenedioxy-*N,N*-dimethylamphetamine (MDMMA) and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB). Ring substituted ethoxy phenethylamines with the same side chain are substanes with an isobaric relationship to these controlled drug substances. All have molecular weight of 207 and major fragment ions in their electron ionization mass spectra at *m/z* 72 and 135/136. All three methylenedioxyphenethylamines were resolved from the ethoxyphenethylamines by means of capillary gas chromatography employing an Rxi-50 stationary phase. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the secondary amines were analysed with GC-MS. The mass spectra for these derivatives were significantly characteristic. The resulting unique fragment ions facilitated specific side chain identification. Perfluoroacyl derivatives displayed reasonable resolution on a non-polar stationary phase such as Rtx-1. GC-IRD analyses produced structure-IR spectra associations that were employed for the discrimination of the three target drugs (MDEA, MDMMA and MBDB) from the other nine ring substituted ethoxyphenethylamine regioisomers

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Anal Chim Acta 2010 **670** (1-2) 63

Validation of an analytical method for analysis of cannabinoids in hair by headspace solid-phase microextraction and gas chromatography-ion trap tandem mass spectrometry

The determination of Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) in samples of human hair by the development of an analytical approach is described. The approach was based on the combination of headspace solid-phase microextraction (HS-SPME) with gas chromatography linked with mass spectrometry operating in tandem mode (GC-MS/MS). Firstly, a 10 mg aliquot of sample was decontaminated using petroleum ether, deionized water and dichloromethane (2 ml of each solvent), for 10 min under

sonication and then digested in alkaline solution (1 mol/l NaOH). Variables evaluated were pH, mass of hair, fiber type, extraction temperature, desorption time, ionic strength, pre-equilibrium time and extraction time. In addition, factors influencing the operation of the tandem mode MS/MS were estimated and optimized. Validation of the approach displayed excellent linearity in the range 0.1-8.0 ng/mg, with regression coefficients better than 0.994. Precision was appraised using two different concentrations (upper and lower limits of the linear range), and RSD values were between 6.6 and 16.4%. Absolute recoveries (measured in triplicate) were in the range 1.1-8.7%, and limits of detection and quantification were 0.007-0.031 ng/mg and 0.012-0.062 ng/mg, respectively. The LOQ for THC (0.062 ng/mg) was below the cut-off value (LOQ 0.1 ng/mg) determined by the Society of Hair Testing (SOHT), the Society of Toxicological and Forensic Chemistry (STFCh) and the Societe Francaise de Toxicologie Analytique (SFTA). The optimized SPME approach was employed in analysis of hair samples from cannabis drug users. It demonstrated that CBN and CBD were present in all samples

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J Chromatogr B 2010 **878** (11-12) 903
The measurement of Ecstasy in human hair by triple phase directly suspended droplet microextraction prior to HPLC-DAD analysis
The determination of Ecstasy, (MDMA; (3,4-methylendioxy-*N*-methylamphetamine) in hair samples has been achieved by the development of a novel pre-concentration technique, triple phase suspended droplet microextraction (SD-LPME) and liquid chromatography-photodiode array. MDMA in hair was digested and subsequently extracted. Influencing conditions were investigated and procedure evaluated. Following optimization, MDMA was enriched by factor 98.11. Linearity (r = 0.9921), was produced in the range of 10-15,000 ng/ml and detection limit was 0.1 ng/ml

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Forensic Sci Int 2010 **198** (1-3) 62
Determination of a new designer drug, *N*-hydroxy-3,4-methylenedioxymethamphetamine and its metabolites in rats using ultra-performance liquid chromatography-tandem mass spectrometry
Recently, a new designer drug has appeared, namely, *N*-hydroxy MDMA (*N*-OH MDMA), an *N*-hydroxy analogue of 3,4-methylendioxymethamphetamine (MDMA). To date, very little data is available to the metabolic and pharmacological properties of *N*-OH MDMA. However, it has been reported that the *N*-demethyl analogue, *N*-hydroxy-3,4-methylenedioxyamphetamine (*N*-OH MDA), is mainly metabolized to MDA in rats. The determination of *N*-OH MDMA and its metabolites in biological samples has been achieved by development of an analytical approach. In addition, the metabolic properties of *N*-OH MDMA in rats have been studied. *N*-OH MDMA and its *N*-dehydroxy and *N*-demethyl metabolites (MDMA, *N*-OH MDA and MDA) have been determined in rat plasma, urine and hair samples were determined by ultra-performance LC (UPLC)-MS/MS following i.p. administration of *N*-OH MDMA to pigmented hairy rats at 5mg/kg/day for 10 days. Hair was extracted by 1 hour sonication and overnight soaking in 5M hydrochloric acid-methanol (1:20). A solid-phase extraction procedure was employed to purify the plasma, urine, and hair extract samples. *N*-OH MDMA in the samples could be precisely analyzed if an alkaline environment was avoided. *N*-OH MDMA disappeared very quickly from rat plasma (< 15 min) and urine (< 10 hours). Most of the *N*-OH MDMA was excreted in the rat urine as MDMA and MDA in 72 hours. In hair collected 4 weeks following the first administration, *N*-OH MDMA (0.03 ng/mg) and *N*-OH MDA (0.13 ng/mg) were clearly detected as well as MDMA (149 ng/mg) and MDA (52 ng/mg). The described approach should be useful for the analysis of *N*-OH MDMA and its metabolites in biological samples

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J Anal Toxicol 2010 **34** (4) 229
Development and validation of a high-performance liquid chromatography method for the evaluation of niflumic acid cross-reactivity of two commercial immunoassays for cannabinoids in urine
In Greece, a nonsteroidal, anti-inflammatory drug routinely prescribed is niflumic acid. It was noted that niflumic acid cross-reacts with cannabinoids in a kinetic interaction of microparticles in a solution (KIMS) immunoassay. This effect was not observedwith an enzyme multiplied immunoassay technique (EMIT) immunoassay. A high-performance liquid chromatographic technique has been developed and validated to evaluate niflumic acid cross-reactivity in two commercial immunoassays for cannabinoids in urine. Niflumic acid standards and in urine specimens produced by subjects receiving niflumic acid were employed. Thirteen niflumic acid concentrations were prepared from standards in drug-free urine ranging from 1.25 to 1000 µg/ml. When the concentration of niflumic acid was above 2.5 µg/ml, the standards produced presumptive positive cannabinoids results if analyzed by the KIMS immunoassay. However, none of the prepared standards produced a false-positive cannabinoid result when analyzed by the EMIT immunoassay. A 50 ng/ml cut-off for cannabinoids was employed and all 55 urine specimens collected from the 5 subjects who participated gave negative results by the EMIT and false-positive by the KIMS immunoassay. Therefore, it is evident that KIMS is more vulnerable to cross-reactions between cannabinoids and niflumic acid compared with EMIT. Consequently, all positive screening tests for cannabinoids produced by KIMS require confirmation with another technique

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Forensic Sci Int 2010 **198** (1-3) 28
Utilization of a detection level of 25 ng/ml for cannabinoids in urine using a CEDIA THCPLUS immunoassay: Application of this cut-off to urines of school children
In New Zealand, cannabis is the most widely used illicit drug. Approximately 4 years ago, schools started introducing drug screening programmes in order ascertain a pupil's likely cannabinoid use. Specimens have been screened for the presence of cannabinoids by means of CEDIA immunoassay, at a cut-off of 50ng/ml as directed by the AS/NZS 4308:2001 standard. However, results reported as not detected (< 50ng/ml) often did not match the pupil's confessed cannabis use. Therefore a lower cut-off of 25ng/ml was employed with this immunoassay. This cut-off was only employed for non-evidential analyses. Stored specimens were analysed over two time periods. Two thousand, three hundred and fifty-nine urine samples were screened for cannabinoids of which 130 had a value between 25 and 49ng/ml. Sixty positive samples were randomly selected for confirmation by GC-MS and the presence of THCCOOH was confirmed in all. Subsequently, 760 specimens were collected over a later time period. Of these, 48 had an immunoassay value of 25-49 ng/ml and all were confirmed positive for THCCOOH by GC-MS. Therefore, the CEDIA® THCPLUS immunoassay may be employed to screen for the presence of urinary cannabinoids using a 25ng/ml cut-off. The use of this cut-off should limit the occurrence of false negative cannabinoid screening results. A lower cut-off is particularly important for school children so that remedial action, following a positive result, may limit the adverse outcomes, for example, dependence and impairment of achievements

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Forensic Sci Int 2010 **199** (1-3) 18
GC-IRD methods for the identification of some tertiary amines related to MDMA
Investigations were performed into seven tertiary amines which have an isobaric or regioisomeric relationship with 3,4-MDMA and its regioisomer; 2,3-MDMA. Direct confirmatory data was achieved with gas chromatography with infrared detection (GC-IRD). Compounds investigated were three ring substituted regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone, two ring regioisomers of *N,N*-dimethyl-2-(methoxymethylphenyl)ethanamine in addition to *N,N*-dimethyl-2-(2,3- and 3,4-methylenedioxyphenyl)ethanamine. The major mass spectral fragments of all of these unique isomers present at equivalent mass and all have equal molecular weight. Therefore, gas chromatography with mass spectrometry detection (GC-MS) is incapable of providing sufficient information for the confirmation of identity of any one of these isomers to the exclusion of the other analytes. The infrared spectra for these compounds facilitate identification of any one of these amines and this is achieved without the aid of chemical derivatization. IR spectra allowed division of the studied compounds into four groups depending on their absorption bands in the region 2700-3100/cm. Furthermore, analytess with different ring substitution pattern within each group may be distinguished by several bands in the 700-1700/cm region. These regioisomeric compounds were well resolved by GC on an Rtx-1 stationary phase. The vapor-phase infrared spectra clearly provides definition within this set of compounds

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Rapid Commun Mass Spectrom 2010 **24** (16) 2357
Determination of four thiophenethylamine designer drugs (2C-T-4, 2C-T-8, 2C-T-13, 2C-T-17) in human urine by capillary electrophoresis/mass spectrometry
An analytical procedure for the simultaneous determination in human urine of four thiophenethylamine designer drugs (2C-T series) is reported. The quantitative analysis was performed by capillary electrophoresis with mass spectrometric detection (CE/MS), using 2,5-dimethoxy-4-methylthiophenethylamine-D_4 (2C-T-D_4) as internal standard. In order to minimize interferences with matrix components and to preconcentrate target analytes, solid-phase extraction (SPE) was introduced in the method as a clean-up step. The method was validated according to international guidelines. The data for accuracy and precision were within required limits. Calibration curves were generated over the range from 10 to 500 ng/ml and correlation coefficients always exceeded 0.997. The method was demonstrated to be specific, sensitive, and reliable for the analysis of these derivatives in urine samples

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J Anal Toxicol 2010 **34** (4) 196
Concentrations of ^{9}-tetrahydrocannabinol and 11-nor-9-carboxytetrahydrocannabinol in blood and urine after passive exposure to cannabis smoke in a coffee shop
Following passive exposure to cannabis smoke under real-life conditions, cannabinoid concentrations in blood and urine were determined. Healthy volunteers (n = 8) were exposed to cannabis smoke for 3 hours in a well-attended coffee shop in Maastricht, Netherlands. Before exposure, blood and urine samples was obtained from each volunteer. Following exposure, blood and urine samples were taken 1.5, 3.5, 6, and 14 hours and 3.5, 6, 14, 36, 60, and 84 hours respectively. Analyses were performed for Δ^9-tetrahydrocannabinol (THC), 11-nor-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) by gas chromatography-mass spectrometry (GC-MS) and immunoassay screening for cannabinoids. All volunteers were found to have absorbed THC. However, the detected levels were quite small. All urine samples failed to produce immunoassay results above the cutoff concentration of 25 ng/ml. Quantitative GC-MS urinalysis demonstrated concentrations of THC-COOH up to 5.0 before and 7.8 ng/ml after hydrolysis. THC was detected in trace amounts close to the limit in the first two blood samples after initial exposure (1.5 and 3.5 hours). After 6 hours blood samples, THC was not detectable. THC-COOH was detectable after 1.5 hours and was still present in 3 out of 8 blood samples after 14 hours in concentrations between 0.5 and 1.0 ng/ml

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J Anal Toxicol 2010 **34** (4) 216
Pharmacokinetic properties of ^{9}-tetrahydrocannabinol in oral fluid of occasional and chronic users
Saliva presents a non- invasive and easily obtainable matrix for detecting drugs drivers. Currently, commercial kits are available where cannabis identification is a major challenge. An understanding of tetrahydrocannabinol (THC) pharmacokinetics in oral fluid has been performed. Analysis of oral fluid demonstrated no significant differences between 12 occasional and 12 chronic users smoking a standardized cannabis joint with the exception of the maximum concentrations in the first samples (occasional users, 397-6438 ng/g; chronic users 387-71,747 ng/g). THC was detectable in all samples with medians in the last samples (8 hours) of 6.3 in occasional and 11.3 ng/g in chronic users. For both groups, elimination half-life was 1.6 +/- 0.4 hours. A series of specimens was obtained over a period of 8 hours without actual drug use representing a later elimination phase. Only 4.3% of oral fluid samples were negative for THC despite positive serum. Conversely, 24.1% of serum samples were negative despite positive oral fluid. This substantiates that THC is detectable for longer in oral fluid than in serum. Oral fluid-to-serum ratios were 0.3 to 425 (median 16.5) and with no difference between chronic and occasional users. The large inter- and intraindividual variability noted impedes reliable estimation of THC serum concentrations from oral fluid data with this collection device

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J Anal Toxicol 2010 **34** (2) 110
Lack of -hydroxybutrate prevalence among an urban emergency department population (Letter)
No abstract available

11 Narcotics

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Forensic Sci Int 2010 **198** (1-3) 118
A reduction in blood morphine concentrations amongst heroin overdose fatalities associated with a sustained reduction in street heroin purity
Between 1/1/1998-31/12/2006, the NSW Department of Forensic Medicine autopsied 959 consecutive cases of heroin overdose. Samples were analyzed to determine the effects of a sudden and sustained reduction in heroin purity on the toxicology of heroin overdose. Over the study period, there was a significant reduction in blood morphine concentration (β = -0.07), declining from a median of 0.50 mg/l in the years 1998-2000 prior to 0.40mg/l in the period 2001-2006. Whereas there was no significant change in the proportion of cases positive for alcohol, the proportions of benzodiazepine (OR 1.11) and methadone (OR 1.12) positive cases increased across time. After controlling for these factors (β = -0.07), the decline in blood morphine concentrations remained significant. When investigating toxic and lethal morphine concentrations, the overdose toxicology influences of changes in the opioid street market should be considered

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J Anal Toxicol 2010 **34** (3) 149
A fast screening procedure for ketamine and metabolites in urine samples with tandem mass spectrometry
Ketamine and metabolites in urine samples were screened by development of an electrospray tandem mass spectrometry (MS-MS) approach. Ketamine-d_4 and norketamine-d_4 were employed to spike urine samples and extracted with 0.5 ml ethyl acetate before analysis with triple-quadrupole MS-MS. Each sample took 1.5 min for analysis. Limit of detection was 0.1 ng/ml for ketamine, norketamine, and dehydronorketamine (DHNK). Carryover rate was less than 0.06%. Within-run and between-run precision for ketamine, norketamine, and DHNK at three different concentrations (40, 75, and 125 ng/ml) was between 2.1 and 8.2%. Within-run and between-run accuracy, presented as % bias, was between -5.9 and 2.7%. Seventy-six urine samples were screened with ELISA and gas chromatography-mass spectrometry (GC-MS). GC-MS was employed as the reference method. When ketamine, norketamine, and DHNK were monitored at cutoff concentration of 100 ng/ml, there were 21 positive, 45 negative, 7 false-negative, and 3 false-positive results. Two hundred and forty-three samples was screened with MS-MS and analyzed with GC-MS. There were 74 positive, 163 negative, 6 false-positive, and no false-negative results. The MS-MS procedure is accurate, efficient, and suitable for use as a high-throughput screening method for ketamine and metabolites

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Forensic Sci Int 2010 **198** (1-3) 31
Chemical analysis of synthetic cannabinoids as designer drugs in herbal products
Forty-six herbal products that are currently distributed on the illegal drug market in Japan due to their expected narcotic effects were analysed. Forty-four were found to contain several synthetic cannabinoids. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) analyses indicated that most of the products contained two major synthetic cannabinoids: (1*RS*,3*SR*)-3-[2-hydroxy-4-(2-methylnonan-2-yl)-phenyl]cyclohexan-1-ol, renamed cannabicyclohexanol with the agreement of Pfizer Inc., and/or 1-naphthalenyl(1-pentyl-1*H*-indol-3-yl)methanone, named JWH-018. Oleamide (*cis*-9,10-octadecenoamide), an endogenous cannabinoid, was detected in 7 products. Two synthetic cannabinoids were identified as minor components in some products. The first was (1*RS*,3*SR*)-3-[2-hydroxy-4-(2-methyloctan-2-yl)phenyl]cyclohexan-1-ol, which is named CP-47,497 and is a homolog of cannabicyclohexanol. The second was 1-naphthalenyl(1-butyl-1*H*-indol-3-yl)methanone, which is named JWH-073 and is a homolog of JWH-018. These synthetic cannabinoids have been reported as possessing cannabimimetic activity. The concentrations of cannabicyclohexanol, JWH-018 and oleamide in the products ranged from 1.1 to 16.9 mg/g, 2.0 to 35.9 mg/g and 7.6 to 210.9 mg/g, respectively. However, they showed considerable variation

12 Forensics

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Forensic Sci Int 2010 **198** (1-3) 39
Simultaneous screening for and determination of 128 date-rape drugs in urine by gas chromatography-electron ionization-mass spectrometry
Date-rape drugs (DRDs) are employed to incapacitate victims to facilitate rape and/or robbery. A wide variety of substances is employed. Their low concentrations in body fluids and frequent delay to clinical examination impedes screening for the presence of these drugs. Detection of the drug used to facilitate a sexual assault is very important evidence of a committed crime. Consequently, an investigation has been conducted to develop a simple GC-EI-MS screening procedure for date-rape drugs in urine. Target analytes were isolated by solid-phase extraction of 2 ml urine samples and then derivatized with BSTFA+1%TMCS. For all compounds, detection was based on full-scan mass spectra. In addition, for each compound one ion was chosen for further quantification. This approach facilitated simultaneous screening, detection and quantification of 128 compounds from different groups (number of compounds): opioids (20), amphetamines (11), GHB and related products (3), hallucinogens (9), benzodiazepines (18), antihistamines (9), antidepressants (14), selective serotonin-reuptake inhibitors (4), antipsychotics (7), barbiturates (7), other sedatives (5), muscle relaxants (2) and other drugs (19). The technique may be easily expanded to include additional compounds. The procedure appeared appropriate for screening for the target DRDs. This approach was successfully employed in the analysis of authentic urine samples collected from victims of rapes and other crimes in routine casework

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Forensic Sci Int 2010 **198** (1-3) 7

Quality assurance in road traffic analyses in Switzerland

For many years, the Swiss Federal Roads Office (FEDRO) has authorized the Swiss laboratories performing toxicological road traffic analyses. To facilitate the complete quality management concerning road traffic analyses, in 2003 FEDRO signed a contract with the Swiss Society of Legal Medicine (SSLM). A multidisciplinary working group was convened under the title of the "Road Traffic Commission (RTC)". RTC has to organize external quality control, to interpret the results of these controls, to perform audits in the laboratories and to report all results to FEDRO. In addition, the working group may be directed to special tasks by FEDRO. An independent organization, the Swiss Center for Quality Control (CSCQ) in Geneva manages the external quality controls in the laboratory. All tested drugs and psychoactive substances are catalogued in a federal instruction. So-called 'zero tolerance substances' (THC, morphine, cocaine, amphetamine, methamphetamine, MDMA and MDEA) and their metabolites rquire testing annually whereas other substances (benzodiazepines, zolpidem, phenobarbital, etc.) periodically. Recent results demonstrate that all laboratories are generally within the confidence interval of +/-30% of the mean value. When non-conformities arise, measures have to be taken immediately and reported to the working group. External audits are performed triennially but accredited laboratories may combine this audit with the approval of the Swiss Accreditation Service (SAS). During the audits, a special checklist filled in by the laboratory director is assessed. Non-conformities necessitate correction. Whilst establishing the new legislation, RTC had an opportunity of advising FEDRO. In collaboration with FEDRO, RTC and hence SSLM work actively to improve quality assurance in road traffic toxicological analyses

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Forensic Sci Int 2010 **199** (1-3) 50

Automated toxicological screening reports of modified Agilent MSD Chemstation combined with Microsoft Visual Basic application programs

By employing total ion chromatogram (TIC) and mass spectroscopy in normal mode, Agilent GC-MS MSD Chemstation offers automated library search report for toxicological screening. In biological specimens such as blood or urine, numerous peaks appear in the chromatogram. Frequently, large migrating peaks eclipse small peaks of interest. Furthermore, target analyte peaks with low abundance often provide the wrong library search result or low matching score. Consequently, retention time and mass spectrum of all the peaks require checking to ascertain their are relevance. Inevitably, such repeated actions may appear monotonous and time-consuming to toxicologists. The MSD Chemstation software operates by employing a number of macro files. These provide commands and instructions on how utilize and extract data from the chromatogram and spectroscopy. The macro files are produced by the compiler of the software. Original macro files may be modified and new macro files may be added to the original software by users. In order to produce results with greater accuracy, with a more convenient approach and to save time for data analysis, new macro files were devised for report generation and inserted into new menus in the Enhanced Data Analysis program. Toxicological screening reports produced by the new macro files are in text mode or graphic mode. They may be generated with three different automated subtraction options. Text reports have brief mode and full mode and graphic reports have the option with or without mass spectrum mode. Matched mass spectrum and matching score for analytes are written into reports by modified library searching modules. An independent application program named DrugMan has been developed. This manages drug groups, lists and parameters that are employed in MSD Chemstation. The incorporation of DrugMan with modified macro modules affords a powerful tool for toxicological analysis and is time efficient

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Forensic Sci Int 2010 **198** (1-3) 58

Urine testing for norcodeine, norhydrocodone, and noroxycodone facilitates interpretation and reduces false negatives

Information to health specialists treating pain patients regarding patient compliance, diversion and concurrent illicit drug use may be provided by urinalysis. An understanding of the results for semi-synthetic opiates is complicated by complex biotransformations of the parent drug to metabolites that are also available commercially and may be abused. However, normetabolites, for example, norcodeine, norhydrocodone and noroxycodone are unique metabolites that are not available commercially. Therefore, the presence of a normetabolite in specimens not containing parent drug provides conclusive evidence that the parent drug was consumed. The prevalence and patterns of the three normetabolites, norcodeine, norhydrocodone and noroxycodone, was investigated in urine specimens of pain patients treated with opiates. Urine specimens were hydrolyzed with β-glucuronidase and subjected to a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) assay. The presence of codeine, norcodeine, morphine, hydrocodone, norhydrocodone, hydromorphone, dihydrocodeine, oxycodone, noroxycodone, and oxymorphone was investigated. The limit of quantitation (LOQ) for these analytes was 50 ng/ml. The research was approved by an Institutional Review Board. Of the total specimens (n = 2654) tested, 71.4% (n = 1895) were positive (>or= LOQ) for one or more of the analytes. The prevalence (%) of positive results for codeine, hydrocodone and oxycodone was 1.2%, 26.1%, and 36.2%, respectively. For norcodeine, norhydrocodone and noroxycodone, the figures were 0.5%, 22.1%, and 31.3%, respectively. In specimens containing a normetabolite, the prevalence of norcodeine, norhydrocodone and noroxycodone in the absence of parent drug was 8.6%, 7.8% and 9.4%, respectively. Between one-third and two-thirds of these specimens did not contain other metabolites that might have originated from the parent drug. Therefore, it is concluded that inclusion of norcodeine, norhydrocodone and noroxycodone is beneficial in the interpretation of opiate drug source and reduces potential false negatives that might occur in the absence of tests for these unique metabolites

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J Anal Toxicol 2010 **34** (4) 204

Comparison of drug concentrations in blood and oral fluid collected with the Intercept sampling device

Drug concentration ratios between oral fluid collected with the Intercept device and whole blood have been investigatedBlood and oral fluid specimens were obtained from patients who had been admitted to acute psychiatric treatment and people suspected of driving under the influence of drugs. Samples were subjected to analysis for drugs of abuse, benzodiazepines, opioids, carisoprodol, and meprobamate. A total of 17 different drugs were discovered in samples of both blood and oral fluid from 59 subjects. Concentration ratios between oral fluid and blood were produced for all cases. Drug concentration ratio distributions were wide for most drugs. They did not permit reliable estimations of drug concentrations in blood using those derived from oral fluid. The median oral fluid/blood drug concentration ratios for the most frequently encountered drugs were 0.036 diazepam, 0.027 nordiazepam, 7.1 amphetamine, 2.9 methamphetamine, 5.4 codeine, 1.9 morphine, and 4.7 tetrahydrocannabinol. For the six predominant drugs, correlation coefficients between drug concentrations in oral fluid and blood ranged from 0.15 to 0.96

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Anal Methods 2010 **2** (5) 552

Molecularly imprinted polymer stir bar sorption extraction and electrospray ionization tandem mass spectrometry for determination of 2-aminothiazoline-4-carboxylic acid as a marker for cyanide exposure in forensic urine analysis

For the determination of cyanide poisoning, a stable and quantifiable marker in biological fluids is required. A reliable biological marker for cyanide exposure is deemed to be 2-aminothiazoline-4-carboxylic acid (ATCA) which is a chemically stable urinary metabolite of cyanide. However, endogenous ATCA is always present at low levels. This might result either from metabolism of dietary sources or from normal metabolism of amino acids. Therefore, a selective and sensitive analytical method is required to estimate the endogenous level of ATCA in order to facilitate a diagnosis of cyanide poisoning. Molecularly imprinted polymers (MIPs) were prepared on the surface of a silica stir bar for molecularly imprinted stir bar sorption extraction (MISBSE). Under optimal conditions, the MISBSE could selectively preconcentrate ATCA from urine samples. The binding capacity of one MISBSE stir bar for ATCA was determined to be 35 +/- 3 ng (n = 3). By combining MISBSE with electrospray ionization tandem mass spectrometry (ESI/MS/MS), ATCA was detectable without derivatization at the 400 ng/ml. This novel approach of MISBSE-ESI/MS/MS increased the selectivity and sensitivity of the detection of ACTA in urine samples

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Forensic Sci Int 2010 **198** (1-3) 46

Potential of IRMS technology for tracing -butyrolactone (GBL)

The popularity of γ-hydroxybutyric acid (GHB) is reasonable constant among drug users. However, the consumption of its chemical precursor, γ-butyrolactone (GBL), continues to grow. Conventional analytical facilitate the detection of this substance in various matrices. However, linking a trace and a source still presents a difficult challenge. Unfortunately, several synthesis pathways and chemical precursors exist for the production of GBL. Therefore, its carbon isotopic signature may vary extensively. Consequently, an approach has been developed to analyze the carbon isotopes content of GBL by means of gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS). The δ^{13}C-values of 19 bulk samples purchased worldwide were in the range from -23.1 to -45.8 per thousand (SD < 0.3 per thousand). Moreover, tests on the purification of GBL by distillation has been found to be inconsistent with such a large range of δ^{13}C-values. These are a likely consequence of

the isotopic composition of the organic precursors combined with the kinetic isotope effect associated with the synthesis routes. Inter- and intra-variability measurements of the $\delta^{13}C$-values revealed the great potential of IRMS for discriminating between seizures of GBL and for source determination

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Clin Chem 2010 **56** (4) 575

Automated mass spectral deconvolution and identification system for GC-MS screening for drugs, poisons, and metabolites in urine

Gas chromatography and/or liquid chromatography coupled to mass spectrometry is often employed in systematic toxicological analysis. However, a particular hindrance is identifying analytes of interest from background noise. One full-scan MS run produces a large amount of spectral information. This necessitates the use of automated evaluation of recorded data files. Therfore, an appraisal has been performed of the freeware deconvolution software AMDIS (Automated Mass Spectral Deconvolution and Identification System) for GC-MS-based systematic toxicological urinalysis intended to reduce the time for identification and automate the daily routine workload. Acidic hydrolysis, liquid-liquid extraction, and acetylation was performed on 111 urine samples for GC-MS analysis. Data files from the analyses were assessed manually by an experienced toxicologist and automatically using AMDIS with deconvolution and identification settings previously optimized. A comparison was made of results obtained by manual and AMDIS evaluation. Deconvolution settings for the AMDIS evaluation were successfully optimized to produce the greatest possible number of components. Identification settings were assessed and selected for a compromise between most identified targets and general number of hits. Following optimization of settings, AMDIS-based data analysis was comparable or even superior to manual assessment and reduced the overall analysis time by half. Therefore, AMDIS was demonstrated to be a reliable and powerful tool for daily routine and emergency toxicology. However, AMDIS is only capable of identifying targets present in the user-defined target library. Therefore, unknown compounds that might be relevant in clinical and forensic toxicology will not be indicated

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J Anal Toxicol 2010 **34** (2) 112

Improved oxymorphone detection in postmortem specimens (Letter)

No abstract available

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Forensic Sci Int 2010 **198** (1-3) 110

Deaths involving serotonergic drugs

Drugs active on the serotonin sytem such as tramadol, venlafaxine, fluoxetine, sertraline, citalopram, paroxetine and MDMA appear relatively frequently in cases of death investigated forensically in the state of Victoria, Australia. During 2002-2008, there were 1123 fatalities where one or more of the serotonergic drugs were discovered. By means of pathology, toxicology and police reports, deaths were examined to investigate whether these drugs where contributary factors especially where serotonin toxicity was suspected. In 28 cases, target drugs were detected and the circumstances indicated the probability of serotonin toxicity. Of the 28, 5 cases reported serotonin toxicity and in 23 others it was suspected. Of the seven target drugs, tramadol was most frequently detected and appeared in combination with serotonergic antidepressants. MDMA also appeared relatively commonly and was associated with moclobemide in 4 cases of confirmed serotonin toxicity. Of the other 1095 cases, natural disease, external injury or the misuse of other drugs appeared to cause death. However, 2 reported the incidental contribution of serotonin toxicity

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Anal Chim Acta 2010 **668** (1) 67

Automated determination of diazepam in spiked alcoholic beverages associated with drug-facilitated crimes

The determination of diazepam (a benzodiazepine) in spiked alcoholic beverages was achieved with a multipumping flow system (MPFS) coupled to a photodegradation unit and fluorimetry. The principal attributes of MPFS include high portability, versatility and straightforward automation and control combined with the efficiency and simplicity of photodegradation. The selectivity and sensitivity of fluorimetric detection makes the developed analytical technique a desirable approach and a valuable contribution for the prevention of drug-facilitated crimes (DFC). Drug-facilitated crimes employ the unauthorized administration of strong central nervous system depressant drugs. These are used to incapacitate victims. Drugs identified as being used in DFC are often covertly placed in drinks of potential victims in entertainment places such as night clubs. Five commercial alcoholic beverages (Eristoff, Smirnoff, Bacardi, Dry Gin and Brazilian Cachaca 51) spiked with diazepam were analyzed by the proposed technique. The results demonstrated good agreement with those produced in a comparison using HPLC. Relative deviations ranged from -1.97 and 2.05%. Analysis with a paired t-test, revealed no statistical difference with a confidence level of 95% (n = 5). The detection limit was approximately 2.02 mg/l.

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J Anal Toxicol 2010 **34** (2) 89

Determination of triazolam and -hydroxytriazolam in guinea pig hair after a single dose

The determination of triazolam and α-hydroxytriazolam in guinea pig hair after a single dose of triazolam. has been achieved with a sensitive liquid chromatography-tandem mass spectrometry technique. A single dose of triazolam was administrated intragastrically to 3 groups of 6 guinea pigs at 10, 100, and 500 μg/kg. Before administration, drug-free hair was shaved from their backs. Newly grown hair in the shaved area was collected every seven days after administration. About 20 mg of decontaminated hair was cut into small segments and incubated in 2 ml of phosphate buffer (pH 8.4) at 45 °C overnight. Triazolam-d_4 and α-hydroxytriazolam-d_4 were employed as internal standards, and liquid-liquid extraction was performed with 3 ml of ethyl ether. The sample was separated on an Allure propyl PFP column with a mobile phase of acetonitrile/20 mM ammonium acetate (7:3, v/v). Detection was accomplished with multiple reaction monitoring mode by an API4000 triple-quadrupole tandem mass spectrometer. Limits of detection for triazolam and α-hydroxytriazolam were 1 and 5 pg/mg, respectively. Triazolam and α-hydroxytriazolam were only detectable in the first week. The minimal dosage detection in guinea pig hair was 100 μg/kg. The concentration of triazolam in hair was related to administration dosage and hair color. A higher concentration of α-hydroxytriazolam compared with triazolam was detected in guinea pig hair

13 Alcohol

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J Anal Toxicol 2010 **34** (5) 261

Direct determination of ethyl glucuronide and ethyl sulfate in postmortem urine specimens using hydrophilic interaction liquid chromatography-electrospray ionization-tandem mass spectrometry

The identification and quantification of ethyl glucuronide (ETG) and ethyl sulfate (ETS) as ethanol biomarkers has been achieved with the development and validation of a a hydrophilic interaction chromatography (HILIC)-electrospray ionization (ESI)-ion trap-tandem mass spectrometric approach. This procedure was employed for the analysis of postmortem urine samples. ETG and ETS were separated on a ZIC-HILIC column (150 x 2.1 mm, 3.5 μm) connected to a Thermo Finnigan LCQ Deca Plus liquid chromatographic- tandem mass spectrometric instrument operated in the ESI-selected reaction monitoring mode. Seventy-nine urine case samples were divided into three groups (A, B and C) depending on the ethanol concentration found in blood and analyzed by the developed approach. Group A had postmortem blood ethanol concentrations higher than 200 mg/100 ml; group B had ethanol concentrations in the range 80-200 mg/100 ml; and group C had ethanol concentrations in the range 10-80 mg/100 ml. ETG and ETS exhibited high recoveries of 98-99%, and the HILIC column produced fine, sharp peak shapes and achieved baseline separation in less than 7 min. Both ethanol markers were detected in all groups with overall median concentrations of 100 and 23 mg/l for ETG and ETS, respectively. It may be concluded that the potential for postmortem production of alcohol increased in the low ethanol concentration group because several cases tested negative for both biomarkers in group C. ETG was detected at low concentrations in some cases for which ETS tested negative. ETS remains stable even after being subjected to many conditions. However, the use of ETS as sole evidence of alcohol ingestion may result in a false-negative result, as was evidenced in groups A and C in the present results. ETG was deemed a more reliable biomarker of ethanol presence. It is necessary to determine both ethanol biomarkers in heavily putrefied cases and where the ethanol concentration in postmortem blood is low

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Alcohol Alcohol 2010 **45** (3) 252

Screening and brief intervention for risky alcohol consumption in the workplace: Results of a 1-year randomized controlled study

The effectiveness of brief alcohol intervention on hazardous and harmful drinking in the 12-month period after a voluntary alcohol screening was investigated. Staff at a large transport company who presented to the occupational health services for a routine health and lifestyle check-up were offered alcohol screening by means of self-report (the Alcohol Use Disorders Identification

Test-AUDIT) and a biomarker (carbohydrate-deficient transferrin in serum-CDT). Employees screening positive for the AUDIT and/or CDT were randomized to a brief or comprehensive intervention group or to a control group. Subsequently, an identical follow-up session was performed after 12 months. Of 990 employees (68% men) that volunteered for the alcohol screening, 194 (20%) tested positive for the AUDIT and/or CDT. One hundred and fifty-eight (81%) subjects attended the follow-up. The frequency of positive screening results at baseline/follow-up were 51%/23% for the AUDIT ($P < 0.0001$) and 58%/34% ($P < 0.0001$) for CDT. No significant differences were noted between the brief and comprehensive intervention groups nor between the intervention groups and the control group. Results indicate that alcohol screening and brief intervention carried out in connection with routine health and lifestyle examinations in the workplace may be effective in reducing alcohol consumption. In addition, the similar outcomes of the intervention groups and the control group indicates that alcohol screening alone might cause a reduction in drinking. In addition, at least some of the positive effect may be explained by regression towards the mean

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J Anal Toxicol 2010 **34** (2) 84

Levels of ethyl glucuronide and ethyl sulfate in oral fluid, blood, and urine after use of mouthwash and ingestion of nonalcoholic wine

An investigation has been conducted into the concentrations of ethyl glucuronide (EtG) in oral fluid and both EtG and ethyl sulfate (EtS) in blood and urine following intense use of mouthwash and ingestion of nonalcoholic wine, which are proven to contain 3 mg/l EtG, 1.5 mg/l EtS, and 0.2 g/l ethanol. In a controlled experiment, 12 subjects ingested nonalcoholic wine and produced urine samples negative for EtG but positive for EtS (C_{max} 2.15 mg/l). Four subjects used mouthwash but all were negative for EtG and EtS in urine. All samples of oral fluid were negative for EtG and all samples of blood were negative for both EtG and EtS. Data demonstrate that ingestion of EtG and EtS as components of nonalcoholic wine results in the detection of only EtS in urine, indicating superior bioavailability of orally ingested EtS compared with EtG. This possibility of false-positive EtS results in urine following ingestion of nonalcoholic wine is an important consideration when using EtG and EtS as markers for alcohol relapse. In addition, results demonstrated that a positive EtG or EtS result following accidental alcohol exposure is unlikely in blood and oral fluid

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Sensor Actuator B Chem 2010 **147** (2) 676

Fiber-optic bio-sniffer (biochemical gas sensor) for high-selective monitoring of ethanol vapor using 335 nm UV-LED

Continuous monitoring of gaseous alcohol with high sensitivity and high-selectivity was achieved by development of a fiber-optic bio-sniffer (biochemical gas sensor). The bio-sniffer employs alcohol dehydrogenase (ADH) for recognition of ethanol. The enzyme usually loses activity in the gas phase. To circumvent this issue, a flow-cell was employed to refresh and wet the enzyme immobilized membrane with circulating phosphate buffer (PB). Ethanol vapor is measured by means of the fluorescence of nicotinamide adenine dinucleotide (NADH), which results from an enzymatic reaction in the flow-cell. To facilitate production of a simplified system suitable for on-site deployment, a high-intensity ultraviolet light emitting diode (UV-LED) was employed as an excitation source. The bio-sniffer performance was assessed using a standard gas generator. Operating parameters of the bio-sniffer were optimized by investigating the fluorescence spectrum and the dependence of fluorescence characteristics on flow-rate of PB. The bio-sniffer was verified to be suitable for continuous gas monitoring and the calibration range was 0.30-300 ppm. High-selectivity because of the specific activity of enzyme was also validated. Therefore, the ADH bio-sniffer is propsed to be a valuable addition to breath analysis or medical screening applications

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Traffic Inj Prev 2010 **11** (2) 123

Abstinence monitoring of suspected drinking drivers: Ethyl glucuronide in hair *versus* CDT

Ethyl glucuronide (EtG) was determined in the hair of subjects who had self-reported not consuming alcohol. Results were reviewed and compared with carbohydrate-deficient transferrin (CDT) blood tests (by immunochemistry and high-performance liquid chromatography [HPLC]). A retrospective study was performed on 154 people whose fitness to drive was necessary to assess following the suspicion of relevant alcohol problems. Abstinence in all subjects was disproved when EtG was detected in 55% of the hair samples. In addition, in two thirds (67%) of these cases, alcohol consumption was demonstrated to be excessive (EtG values > 30 pg/mg). Of the EtG-positive subjects 54% and 82% had CDT values within the reference range by immunochemistry and HPLC, respectively. Thirty-nine percent of the EtG-negative subjects had increased immunochemical CDT values. By contrast, 96% had HPLC CDT values within the normal range. Therefore, EtG analysis in hair is effective for assessing fitness to drive in suspected drinking drivers. When compared with CDT values it facilitates direct and unequivocal abstinence monitoring over a period of several months, depending on the length of the hair

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Forensic Sci Int 2010 **198** (1-3) 23

Effect of bleaching on ethyl glucuronide in hair: An *in vitro* experiment

Recently, ethyl glucuronide in hair (HEtG) has achieved great interest because of its high sensitivity and specificity in the diagnosis of chronic alcohol abuse. However, due to its high polarity hydrophilicity, strong hair treatment followed by a shampooing might result in the removal and/or degradation of this analyte from the hair matrix. The effects of hair bleaching to modify HEtG results was investigated. Following informed written consent, 38 hair samples from teetotalers (n = 5), social drinkers (n = 4) and heavy drinkers (n =21) were collected and divided longitudinally into four aliquots. One aliquot was not treated and was processed following the method routinely used in our lab for the determination of HEtG (double washing with methanol/dichloromethane, overnight incubation in water, and LC-MS/MS analysis, LLOQ: 3pg/mg). To the three remaining aliquots, a commercially available bleaching solution was applied in accordance with the manufacturer's instructions. One aliquot was analyzed by the same procedure used for the untreated sample. The other two were submitted to a purification step with two different SPE cartridges (aminopropyl and dimethyl butylamine) before LC-MS/MS analysis. HEtG levels in the untreated samples from social drinkers and heavy drinkers ranged from 7.7 to 149.0 pg/mg and all samples from teetotalers tested negative. Treated samples not subjected to SPE extraction and with aminopropyl cartridges showed relevant ion suppression for both EtG and D_5-EtG (IS) signals. Samples treated with the bleaching solution and extracted with dimethyl butylamine cartridge reduced ion suppression (less than 35%). They demonstrated that EtG, after a strong treatment such as bleaching, is completely eliminated. However, it is unclear whether the mechanism involved is chemical degradation or physical removal from a damaged matrix. Due to the highly hydrophilic character of the compound, the latter mechanism is proposed as the more likely. In addition, bleaching solutions might result in heavy ion suppression that may be avoided by using an SPE purification before instrumental analysis

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Electrophoresis 2010 **31** (7) 1227

Analysis of the alcohol biomarker phosphatidylethanol by NACE with on-line ESI-MS

Phosphatidylethanol (Peth), a group of aberrant phospholipids formed in cell membranes in the presence of ethanol, has been recently proposed as biomarker of chronic alcohol abuse. The aim of this study was to develop a new analytical method, based on NACE online coupled with a mass spectrometer for the analysis of Peth in blood. For this purpose an ion-trap mass spectrometer equipped with an orthogonal ESI source operating in negative ion mode was used. An alkaline solution of ammonium acetate 5 mM (pH 9) in water/methanol (MeOH) (80:20 v/v) was delivered as coaxial sheath liquid. All experiments were performed using an uncoated fused-silica capillary (90 cm x 75 µm id). The effects of variable percentages of ACN, MeOH, 2-propanol, dichloromethane, along with variable concentrations of ammonium acetate were investigated for the separation of Peth. Collectively, a separation medium composed of ACN (45% v/v), 2-propanol (20% v/v), dichloromethane (20% v/v), MeOH (10% v/v), water (5% v/v), and ammonium acetate (25 mM) was chosen. The estimated LOD was 0.1 µM, while LOQ was 0.4 µM. Within-run (intra-day) and between-run (inter-day) precision was always lower than 15%. The method proved to be robust and reliable. The MS detector allowed the simultaneous identification of several Peth homologues, and the use of an internal standard (phosphatidylbutanol) with similar electrophoretic properties of that of Peth increased quantitation effectiveness

14 Tobacco

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Forensic Sci Int 2010 **199** (1-3) e9

Two UK suicides using nicotine extracted from tobacco employing instructions available on the internet

One of the most easily accessible and commonly abused drugs world-wide is tobacco. However, one of its principal constituents is nicotine and this may

result in serious or fatal overdoses. Fortunately, deliberate ingestion of this dangerous substance appears to be infrequent. Important signs of its consumption are not easily recognised and this may result in fatalities. Two instances of intentional fatal ingestion of nicotine are described. Nicotine was extracted from tobacco using instructions available on the Internet. The first instance was of a male who died in 2008 aged 19. Post-mortem levels of nicotine in blood were 5.5 mg/l and urine > 80 mg/l. The concentration in blood corresponds with the generally recognised fatal level of > 5 mg/l. The primary metabolite of nicotine is conitine. This was found to be 2.5 mg/l in blood and 7.9 mg/l in urine. Another instance was of a 32 year-old male in 1999. The post-mortem concentration of nicotine in his blood was 1.0 mg/l. These 2 cases are believed to be the first suicides in the UK reported in the literature employing nicotine and using instructions obtained from the Internet. The cause of death as nicotine only became apparent some time after death. Consequently, there may be more deaths due to nicotine that go unrecognised. Quantification of nicotine and cotinine levels are rarely conducted because of the wide incidence of smoking. In addition, these instances emphasize the importance of carefully evaluating all evidence at the scene of a sudden and unexplained death including potential clues on home computers

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Hum Exp Toxicol 2010 **29** (5) 385

Nicotine and its metabolites in amniotic fluid at birth—assessment of prenatal tobacco smoke exposure

Seventy-eight pregnant women were screened. Urine was collected prior to delivery, amniotic fluid at birth and neonatal urine and meconium. High-performance liquid chromatography (HPLC) was employed to determine the smoking markers, nicotine and its metabolites cotinine and *trans*-3'-hydroxycotinine (OH-cotinine). Self-reported smoking status during pregnancy determined by means of a questionnaire and this was verified by analysis of maternal urine. Nicotine was detected 80% and nicotine metabolites in 100% of the amniotic fluids. However, the sum of the nicotine metabolites (Sum_{met}) was significantly lower ($p < 0.001$) in amniotic fluid (704 +/- 464 nmol/l) than in meconium (921 +/- 588 nmol/l), neonatal urine (1139 +/- 813 nmol/l) and maternal urine (4496 +/- 3535 nmol/l). There was a good correlation ($p < 0.001$) between concentrations of nicotine metabolites in amniotic fluid and that of other specimen types. Following environmental tobacco smoke (ETS) exposure, nicotine or nicotine metabolites were detectable in maternal and neonatal urine but not in amniotic fluid. Analysis of amniotic fluid at birth permits verification of smoking habits during pregnancy and clearly distinguishes against ETS exposure. However, it is not a suitable technique to differentiate between ETS exposure and non-exposure

15 Homeland Security

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Anal Chem 2010 **82** (12) 5246

Identification of ricin in crude and purified extracts from castor beans using on-target tryptic digestion and MALDI mass spectrometry

Seeds of the castor bean plant contain the toxic protein lectin, ricin. The toxicity of the lectin and the ease with which it may be extracted from seeds make it a potent biological warfare agent. Therefore, there have been extensive studies in the development of analytical techniques which are able to identify the protein both robustly and rapidly. On-target tryptic digestion and MALDI MS was employed to distinguish ricin from bovine serum albumin and three other type 2 ribsome-inactivating proteins (RIPs), abrin, agglutinin (RCA_{120}), and viscumin, using the peptide mass fingerprint. The sequence coverage produced was increased using methanol-assisted tryptic digestion. This approach was especially appropriate for the detection of lectins in complex matrixes. In conjunction with intact protein MALDI mass measurement, a positive identification of ricin (or any of the other RIPs) was accomplished including affirmation of the integrity of the disulfide bond between the A and B chains. This usefulness of this approach was illustrated by the identification of ricin in a typical "crude white powder" of the sort that may be produced illicitly in a clandestine lab. Analysis on the solubilized sample with this technique may be undertaken in around an hour with little sample preparation

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Anal Chem 2010 **82** (10) 4165

Signature-discovery approach for sample matching of a nerve-agent precursor using liquid chromatography-mass spectrometry, XCMS, and chemometrics

The discovery of trace forensic signatures for matching samples of ten stocks of the nerve-agent precursor known as methylphosphonic dichloride (dichlor) has been achieved by employing bioinformatic and chemometric tools on liquid chromatography-mass spectrometry (LC-MS) data. By using a known set of dichlor samples, a software tool primarily used in bioinformatics (XCMS) was employed to comprehensively search and identify candidate LC-MS peaks. Subsequently, these were reduced to a group of 34 impurity peaks. Hierarchal cluster and factor analysis revealed the potential of these 34 peaks to identify their stock source. Only one pair of dichlor stocks was not differentiated from one another. An suitable chemometric protocol for sample matching was variance scaling and signal averaging of normalized duplicate impurity profiles before classification by K-nearest neighbors. With this technique, a test set of seven dichlor samples were all correctly matched to their stock source. Sample preparation and LC-MS approach facilitated the detection of dichlor impurities quantitated in parts-per-trillion (w/w). Interestingly, a common impurity was discovered in all dichlor stocks synthesized by different manufacturers and over a 14-year period. This signature-discovery approach should assist forensic investigations in the event of chemical warfare attacks

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J Chromatogr B 2010 **878** (17-18) 1382

Stable adducts of nerve agents sarin, soman and cyclosarin with TRIS, TES and related buffer compounds - Characterization by LC-ESI-MS/MS and NMR and implications for analytical chemistry

The control pH in solutions employed for chemical, biochemical and biomedical applications is usually achieved with buffering compounds such as TRIS. In addition to providing sufficient buffering capacity and pH stability over time, it is essential that the compound does not react with other constituents of the solution. This is particularly necessary in the field of analytical chemistry where analytes are to be determined quantitatively. However, during the enzymatic hydrolysis of G-type nerve agents sarin, soman and cyclosarin in buffered solution stable adducts of TRIS, TES and other buffer compounds with the nerve agents have been identified. These were identified as phosphonic diesters using 1D ^{1}H-^{31}P HSQC NMR and LC-ESI-MS/MS methods. The reaction rates with TRIS and TES were rapid enough to compete with spontaneous hydrolysis in aqueous solution and to provide considerable amounts (up to 20-40%) of buffer adduct over the course of several hours. A reaction mechanism is suggested; the amino function of the buffer functions as an intramolecular proton acceptor rendering the buffer hydroxyl groups nucleophilic sufficient for attack on the phosphorus atom of the agents. Similar buffer adductswere noted with a range of hydroxyl and amino function containing buffers including TES, BES, TRIS, BIS-TRIS, BIS-TRIS propane, Tricine, Bicine, HEPES and triethanol amine. As a result of this investigation, it is advised that alternative buffers like MOPS, MES and CHES be employed when working with G-type nerve agents especially at higher concentrations and over prolonged times

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J Agric Food Chem 2010 **58** (11) 6600

Simultaneous multiplex detection and confirmation of the proteinaceous toxins abrin, ricin, botulinium toxins, and *Staphylococcus* enterotoxins A, B, and C in food

Highly specific and sensitive techniques are necessary to identify proteinaceous toxins in complex heterogeneous mixtures. Multiplex technology using several antibodies which recognize different epitopes on a toxin facilitates built-in certification within the initial screen. This enhances the credibility of both presumptive positive and negative results. Polyclonal and monoclonal antibodies were obtained for lectins (abrin and ricin), botulinum toxins, and *Staphylococcus* enterotoxins A, B, and C (SEA, SEB, and SEC). Toxins were spiked either individually or mixed into food samples and analyzed following 40-fold dilution. Abrin, botulinum toxin A complex, ricin, and SEB displayed limits of detection in the original food samples ranging from 0.03 to 1.3 μg/ml, from 0.03 to 0.07 μg/ml from 0.01 to 0.1 μg/ml, and from <0.01 to 0.03 μg/ml, respectively. Redundancy of antibodies (i.e. several for each toxin) some recognizing different epitopes or displaying different binding affinities, produced a "fingerprint" for the presence of the toxins and built-in verification. This mitigates against the likelihood of false-positive and false-negative results. Internal controls, including a unique protein were added to guard against variations in dilution. Paramagnetic microspheres expedited the detection of toxins in foods containing particulate matter not suitable with the use of filter plates routinely employed in the wash steps of assays utilising standard polystyrene microspheres

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J Proteome Res 2010 **9** (7) 3647

Identification of *Yersinia pestis* and *Escherichia coli* strains by whole cell and outer membrane protein extracts with mass spectrometry-based proteomics

Whole cell protein and outer membrane protein (OMP) extracts were com-

pared for their ability to identify bacteria against a proteome database. Extracts were prepared from pathogenic and nonpathogenic strains of *Yersinia pestis* and *Escherichia coli* using ultracentrifugation and sarkosyl technique. Subsequently, protein digestion was performed and analysis was by means of liquid chromatography tandem mass spectrometry (MS). Inevitably, whole cell protein extracts contain numerous proteins which reflect the phase of the growth cycle. However, OMPs are frequently associated with virulence in Gram-negative pathogens. These might provide characteristic biomarkers for strain differentiation among bacteria. Mass spectra of bacterial peptides were searched against a constructed proteome database of microorganisms using the SEQUEST algorithm. This was performed in order to appraise the identity and number of unique peptides in each bacterial sample. Data analysis was carried out with in-house BACid software. It estimated the probabilities that an assigned peptide sequence to a product ion mass spectrum was correct. In addition, it employed accepted spectrum-to-sequence matches to provide a sequence-to-bacterium (STB) binary matrix of assignments. Peptide sequences that had been validated, either present or absent in various strains (STB matrices), were visualized as assignment bitmaps. These were analyzed with the BACid module that utilised phylogenetic relationships among bacterial species as part of a decision tree process. The bacterial classification and identification algorithm employs assignments of organisms to taxonomic groups (phylogenetic classification). These are based on an conceptualized scheme that starts at the phylum level and works through the class, order, family, genus, and species to the strain level. The number of unique characteristic proteins resulting from the whole cell method was less than that of the OMP method for both bacteria. Conversely, the degree of differentiation in terms of linkage distance units on a dendrogram with the OMP extract demonstrated similar or significantly greater separation compared with the whole cell protein extract between the sample and correct database match compared with the next nearest neighbor. The nonpathogenic *Y. pestis* A1122 strain employed does not have an available genome. Data analysis produced an equal similarity index to the nonpathogenic 91001 and pathogenic Antiqua and Nepal 516 strains for both extraction methods. Pathogenic and nonpathogenic strains of *E. coli* were correctly identified with both protein extraction techniques. Pathogenic *Y. pestis* CO92 strain was correctly identified with the OMP procedure. In general terms, proteomic MS facilitated the analysis of unique protein assignments for strain differentiation of *E. coli* and *Y. pestis*. The potential of bacterial protein capture by the whole cell protein and OMP extraction methods was confirmed by the data analysis techniques. It demonstrated differences and similarities between the two protein extraction procedures for bacterial identification

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J Chromatogr B 2010 **878** (17-18) 1226
GC-MS and LC-MS analysis of nerve agents in body fluids: Intra-laboratory verification test using spiked plasma and urine samples
Techniques for the identification of unknown nerve agents in human body fluids have been verified. Biological samples were spiked with nerve agents (blood plasma) or with their metabolites (urine) or were left blank. Seven random samples (35% of all samples) were chosen for the validation test. Plasma was analysed with GC-MS for unchanged nerve agents and for regenerated nerve agents after fluoride-induced reactivation of nerve agent-inhibited butyrylcholinesterase. Metabolites were extracted from plasma and urine and analysed by LC-MS. Urinary metabolites and two blank samples could be identified without further measurements. Plasma metabolites and blanks were identified in six of seven samples. The analysis of unchanged nerve agent identified five agents/blanks and a sixth agent after further investigation. During the first screening, a rather noisy baseline impeded the determination of the regenerated agents and provided only five clear findings. To mitigate this effect, sample preparation was extended to include a size exclusion step performed before addition of fluoride This visibly reduced baseline noise and thus improved identification of the two missing agents. Results suggest that verification should be performed by analysis of more than one biomarker to ensure identification of the agent(s).

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Talanta 2010 **81** (4-5) 1568
Tandem capillary column gas chromatography-mass spectrometric determination of the organophosphonate nerve agent surrogate dimethyl methylphosphonate in gaseous phase
The verification of the ubiquitous and versatile chemical and nerve agent simulant, dimethyl methyl phosphonate (DMMP; CAS# 756-79-6), from gaseous samples was accomplished by the development of an approach based on capillary column gas chromatographic-mass spectrometric (GC-MS). This technique was produced to confirm low nanogram DMMP concentrations during testing of a nerve agent detection system. Standard solutions of 1, 5, 10, 50, 100, 500, and 1000 ng/ml DMMP in acetonitrile were utilised. Through 15 calibration curves using the 5 lowest concentrations, coefficient of determination (r^2) values showed a mean of 0.998 (0.992-1.000). Fifteen additional calibration curves similarly including 5 concentrations of DMMP spanning 3 orders of magnitude (1, 50, 100, 500, and 1000 ng/ml) yielded a mean r^2 of 0.997 (0.991-1.000). Sixty-five nitrogen diluted gaseous samples from 1.0 to 10.0 µl in volume were analyzed and concentrations of DMMP ranging from 1 to 1000 ng/ml were verified. An further 35 vapor samples in UHP N_2 ranging in DMMP concentration from 5.8 µg/m^3 to 1.0 mg/m^3 were analyzed by increasing sample volume range to between 10.0 and 100 µl. For gaseous samples with volumes > 1.0 µl, the lowest concentration noted was 5.8 µg/m^3. The procedure detection limit (Appendix B of Title 40 CFR, United States) for 1.0 µl autoinjected standards in acetonitrile was demonstrated to be 0.331 ng/ml. Technique precision for 15 independently analyzed standards of 25 ng/ml had a relative standard deviation of 1.168. This approach exhibited high linearity across a wide range of concentrations. In addition, there was excellent sensitivity and repeatability. It also proved applicable to other lower alkylphosphonates

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Anal Biochem 2010 **401** (2) 211
Detection of residual toxin in tissues of ricin-poisoned mice by sandwich enzyme-linked immunosorbent assay and immunoprecipitation
An approach has been assessed which facilitates the detection of the residual ricin in animal tissues. This includes immunoprecipitation and sandwich enzyme-linked immunosorbent assay (ELISA) to detect ricin in the tissues of intoxicated mice. Monoclonal antibodies (Mabs) 4C13 and 3D74 were employed to assay the whole ricin molecules *via* sandwich ELISA. Mab 4C13 was conjugated with Sepharose 4B to capture ricin or ricin A chain by immunoprecipitation. Following intravenous ricin at the dosage of 5 µg/mouse, animal were euthanized at times after intoxication. Serum, liver, kidney, lung, and intestine were harvested. By employing sandwich ELISA, high levels of ricin were discovered in serum and liver samples at each time point. Therefore, the possibility is presented of determining ricin intoxication by detecting residual amounts in serum. The procedure proved ineffective for analyzing ricin in kidney, lung and intestine. However, tissue samples analyzed by immunoprecipitation did produce positive bands. This suggests that some component(s) of the kidney, lung, and intestine might bind with ricin and so interfere with its binding activity with the coated antibody. Immunoprecipitation might be employed to indicate the presence of ricin in these tissues

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Anal Chem 2010 **82** (9) 3764
Ion chemistry of VX surrogates and ion energetics properties of VX: New suggestions for VX chemical ionization mass spectrometry detection
VX chemical warfare agent surrogates representing the amine (triethylamine) and organophosphonate (diethyl methythiomethylphosphonate (DEMTMP)) portions of VX were investigated. Room temperature rate constants and product ion branching ratios were measured for the reactions of numerous positive and negative ions. Furthermore, theoretical calculations of the proton affinity, fluoride affinity, and ionization potential of VX and the simulants were performed. Results demonstrate that many proton transfer reactions are rapid and that the proton affinity of VX is near the top of the scale. Using chemical ionization mass spectrometers, many proton transfer agents should detect VX both selectively and sensitively. Charge transfer with NO^+ should also be sensitive and selective because the ionization potential of VX is small. Surrogate studies confirm these trends. Estimates of limits of detection for commercial CIMS instruments are 80 pptv whereas those of research grade equipment are 5 ppqv.

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Anal Methods 2010 **2** (6) 661
Gas chromatography-mass spectrometric analysis of trimethylsilyl derivatives of toxic hydrolyzed products of nerve agent VX and its analogues for verification of chemical weapons convention
The zwiterionic character character of the hydrolyzed product of nerve agent VX [*S*-2-(*N,N*-diisopropylaminoethyl) methylphosphonothiolate (EA-2192)] has been reported to impair the formation of trimethylsilyl (TMS) derivative. However, described herein is the synthesis and analysis the TMS derivative of EA-2192 and its analogues. The issue of non-detectability in GC-MS analysis was discovered to be due to their condensation and subsequent decomposition on the GC column. An optimized temperature program, commencing at 175 °C column temperature, favours partitioning of TMS derivatives of EA-2192 and its analogues into the mobile phase and this enables their detection because it reduces their on-column condensation and decomposition. Excellent reproducibility (< 1% RSD) of TMS derivatives of *S*-2-(*N,N*-dialkylaminoethyl) alkylphosphonothiolates was accomplished with GC-MS analysis and a temperature program of 175 °C (2 min) at 10 °C/min to 300 °C (5 min). Employing the optimized conditions, LODs ranged from 8-10 µg/ml for different analytes at a split ratio of 1:10

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J Chromatogr B 2010 **878** (17-18) 1297
Mass spectral characterization of organophosphate-labeled, tyrosine-containing peptides: Characteristic mass fragment and new binding motif for organophosphates
Organophosphorus agent (OP)-tyrosine adducts on 12 different proteins labeled with six different OP have been identified. Labeling was accomplished with up to 40-fold molar excess of OP at pH 8-8.6 to treat pure proteins. Following digestion of OP-treated proteins with trypsin, resulting peptides were separated by HPLC. Mass spectrometry was employed to investigate fragmentation patterns for 100 OP-peptides labeled at tyrosine. Characteristic ions at 272 and 244 amu for tyrosine-OP immonium ions were rarely absent from the MSMS spectrum of peptides labeled at tyrosine by chlorpyrifos-oxon. Signature fragments were also noted from parent ions that had been labeled with diisopropylfluorophosphate (216 amu), sarin (214 amu), soman (214 amu) or FP-biotin (227, 312, 329, 691 and 708 amu). OP-reactive serines lie in the consensus sequence GXSXG. However, the OP-reactive tyrosines have no consensus sequence but a common feature is the presence of nearby positively charged residues that activate the phenolic hydroxyl group. The most interesting discovery is the recognition of a new binding motif for OP with proteins that have no active site serine. Inevitably, peptides modified with OP are difficult to find in the absence of a radiolabel or tag. Characteristic fragment ions derived from MSMS are important because they identify OP-tyrosine irrespective of the peptide

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J Anal Toxicol 2010 **34** (3) 122
Rapid analysis of Lewisite metabolites in urine by high-performance liquid chromatography-inductively coupled plasma-mass spectrometry
By means of the urinary metabolite 2-chlorovinylarsonous acid (CVAA) and the oxidized metabolite 2-chlorovinylarsonic acid (CVAOA), a high-throughput approach has been developed for determining Lewisite [dichloro(2-chlorovinyl)arsine] exposure. Rapid sample preparation consists of a simple dilution of 400 µl of urine with 40 µl of water and 1 ml of buffer containing an internal standard and brief centrifugation before analysis with high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (ICP-MS). CVAOA and CVAA were eluted isocratically with retention factors of approximately 3.0 and 4.2, respectively, from a reversed-phase polar embedded column with a cycle time of 5 min per sample. The dynamic reaction cell, ordinarily employed to remove polyatomic isobaric interferences, was not necessary for ICP-MS analysis following the resolution of chloride from arsenical peaks of interest. This technique was utilised to detect CVAA and CVAOA in the urine of a rat administered Lewisite up to 24 h after exposure. The protocol exhibited linearity over at least three orders of magnitude and had a detection limit of 1.3 µg/l as CVAA (1.4 µg/l CVAOA). Relative standard deviations for quality control samples ranged from 3 to 6%. This approach was sensitive and selective. There were no false positives in 100 different urine samples collected from individuals with no known exposure to Lewisite. In an 8-h day, up to 96 samples could be analyzed

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J Chromatogr B 2010 **878** (17-18) 1253
Liquid chromatography tandem mass spectrometry applied to quantitation of the organophosphorus nerve agent VX in microdialysates from blood probes
VX (*O*-ethyl-*S*-[2(di-isopropylamino)ethyl] methylphosphonothiolate) is an organophosphorus (OP) nerve agent with low volatility. Therefore, exposure would probably arise *via* percutaneous absorption. Microdialysis was employed as an approach to study percutaneous poisoning by VX in samples collected from microdialysis probes implanted into a blood vessel of anesthetised guinea pigs. Subsequently, liquid chromatography tandem mass spectrometry (LC-MS-MS) method using positive electrospray ionisation (ESI) was employed to quantitate VX in microdialysate. This approach was developed as a modification of a LC-MS-MS technique previously devised for the analysis of dermal microdialysates. The adaptation enhanced the sensitivity of the technique, facilitating quantitation of the trace levels of VX in blood microdialysates, over the range 0.002-1 ng/ml, with linear calibration. Quantitative results were employed to determine the time course of VX concentrations in the blood of guinea pigs following percutaneous poisoning

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J Chromatogr B 2010 **878** (17-18) 1290
Development and validation of a sensitive gas chromatography-ammonia chemical ionization mass spectrometry method for the determination of tabun enantiomers in hemolysed blood and plasma of different species
The chiral quantification of the highly toxic organophosphorus compound tabun (*O*-ethyl-*N,N*-dimethylphosphoramidocyanidate, GA) in hemolysed swine blood has been accomplished by the development and validation of a fast, sensitive and easily applicable GC-MS approach to assist in toxicokinetic and toxicodynamic studies. These conditions were best achieved with a GC-MS assay employing positive chemical ionization with ammonia (GC-PCI-MS). Separation was performed on a β-cyclodextrin capillary column (Supelco BetaDex 225) after reversed phase (C18) solid-phase extraction. The limit of detection was 1 pg/ml for each enantiomer (approximately 500 fg on column) and the limit of quantification 5 pg/ml. Following hemolysis, the GC-PCI-MS technique was employed for the quantification of tabun enantiomers in spiked swine blood. It was also applied to spiked plasma of different species including humans

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J Chromatogr B 2010 **878** (17-18) 1320
Comprehensive gas chromatography with time of flight MS and large volume introduction for the detection of fluoride-induced regenerated nerve agent in biological samples
Verification of exposure to nerve agents has been assisted by the recent development of several approaches. These techniques are primarily based on mass spectrometry, for example, the fluoride reactivation technique and the analysis of inhibited phosphonylated butyrylcholinesterase (BuChE). However, the high specificity of the mass spectrometer might also convey a disadvantage. The acquisition mass (from the identity of the analyte) must be known in advance in order to perform the MS analysis in the most sensitive mode. Inevitably, the identity of the nerve agent is not always apparent. Therefore, the mass spectrometer requires operation in scanning mode and this confers lower sensitivity. Improved separation may be achieved with comprehensive GC, or GC x GC. The manifest greater selectivity of GC separation enables mass spectrometry to be conducted in a less specific, scanning mode. Therefore, utilisation of this approach means that the identity of the nerve agent does not have to be known hitherto. To facilitate the detection of lower concentrations and evaluate lower exposure levels, a large volume injection approach was developed allowing sample sizes up to 100 µl. Plasma samples that contained various nerve agents were employed to test the technique. The cholinesterase-bound nerve agent was regenerated by the fluoride reactivation technique. By means of the novel and comprehensive GC-MS procedure nerve agent exposure levels at 1% BuChE inhibition were detectable. This approximates with 70 pg nerve agent/ml. Exposure at these levels are not verifiable with a cholinesterase (ChE) activity assay. In addition, the regenerated nerve agent could be confirmed with the mass spectrum produced by the TOF mass spectrometer. Consequently, full-scan data on the analysis of nerve agents in biomedical samples at relevant exposure levels (1% BuChE inhibition) may be derived. These achieve to a large extent, the current forensic parameters for the analysis of biomedical samples in the context of alleged use of chemical warfare agents

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J Chromatogr B 2010 **878** (17-18) 1433
GC-MS based identification of skunk spray maliciously deployed as "biological weapon" to harm civilians
An investigation was conducted to elucidate the origin of a strong "toxic smell" present in a prominent politician's office, private house and motorcar. As a result of the stench, serious nausea and vomiting had occurred in several individuals. Persons presenting with symptoms of nausea provided urine samples for toxicological analysis. Liquid drops, paper and cotton swabs of an oily substance discovered *in situ* were submitted for analysis. Methanol extracts of the liquid drops were acetylated for gas chromatography/mass spectrometry (GC/MS) analysis in the electron impact mode. Cotton and paper swabs were analysed using headspace-gas chromatography/mass spectrometry (HS-GC/MS). The GC/MS analysis of the acetylated methanol extracts revealed that the major peaks of the chromatogram could be attributed to 2-methylquinoline, 2-quinolinemethanethiol, *S*-2-quinolinemethyl thioacetate, 2-phenylethanethiol, bis(*E*)-2-butenyl disulphide and bis(3-methylbutyl) disulphide. Several volatile sulphur-containing compounds were identified with the HS-GC/MS system. Analysis of the spectra in addition to GC/MS analysis of commercially available skunk secretion facilitated identification of the compounds as those present in the defence spray of skunks.

16 Workplace

Tsatsakis AM, Barbounis MG, Kavalakis M, Kokkinakis M, Terzi I, Tzatzarakis MN// Univ Crete, Sch Med, Forensic Sci & Toxicol Dept, Div Morphology, Ctr Toxicol Sci & Res, POB 1393, GR-71003 Iraklion, Crete, Greece
J Chromatogr B 2010 **878** (17-18) 1246
Determination of dialkyl phosphates in human hair for the biomonitoring of exposure to organophosphate pesticides
The measurement of four dialkyl phosphates (DAPs) in human head hair has been achieved by the development of a novel, simple, fast and sensitive

technique. The dialkyl phosphates, dimethyl phosphate (DMP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP) are non-selective metabolites of the organophosphate pesticides (OPs). A one step methanolic approach was performed to isolate DAPs from the hair matrix. Head hair samples were obtained from the general population and workers exposed to OPs. Samples were analysed using gas chromatography-mass spectrometry (GC-MS) after derivatization with pentafluorobenzylbromide. Recovery of target compounds was estimated at 84.3% for DMP, 116.1% for DEP, 109.0% for DETP and 91.5% for DEDTP. The limits of quantitation (LOQ) and detection (LOD) were 20 and 6 pg/mg for DMP, 10 and 5 pg/mg for DEP and DETP and 5 and 3 pg/mg for DEDTP, respectively. With-run and between-run precision as well as accuracy was dtermined. The percentage of positive hair samples for DMP, DEP, DETP and DEDTP in the general population samples was 63.0%, 96.3%, 66.7%, and 70.4% respectively. Samples from the group with occupational exposure were positive for all dialkyl phosphates analysed. Median concentrations for DMP were 165.0 and 181.7 pg/mg, for DEP were 51.2 and 812.9 pg/mg, for DETP were 54.0 and 660.1 pg/mg, and for DEDTP were 40.0 and 60.6 pg/mg for the general population and the occupationally exposed groups respectively. Significant differences in the levels of the total dialkyl phosphates amongst exposed and not exposed groups were observed ($p < 0.001$). Total ethyl phosphate (DEPs) and DAPs median concentrations were 119.5 and 301.5 pg/mg for the general population group and 1498.8 and 1694.4 pg/mg for the occupationally exposed group respectively

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J Chromatogr B 2010 **878** (17-18) 1257
Revised method for routine determination of urinary dialkyl phosphates using gas chromatography-mass spectrometry
For occupational and non-occupational OP exposure risk assessment, dialkyl phosphates (DAPs), among urinary organophosphorus pesticide (OP) metabolites, have been most frequently measured as a sensitive biomarker. Analysis involved simple liquid-liquid extraction (diethyl ether/acetonitrile), derivatization (pentafluorobenzylbromide, PFBBr) and clean-up (multi-layer column) for gas chromatography-mass spectrometry (GC-MS) analysis starting from 5 ml urine samples. A revised analytical technique has been developed for urinary DAPs with its primary modification addressed at improving the pre-derivatization dehydration procedure. Limits of detection were approximately 0.15 µg/l for dimethylphosphate (DMP), 0.07 µg/l for diethylphosphate (DEP), and 0.05 µg/l for both dimethylthiophosphate (DMTP) and diethylthiophosphate (DETP) in 2.5 ml human urine samples. Within-run precision (percent of relative standard deviation, %RSD) at the DAP levels varying in the range of 0.5-50 µg/l was 6.0-19.1% for DMP, 3.6-18.3% for DEP, 8.0-25.6% for DMTP and 9.6-27.8% for DETP. Between-run precision at 5 µg/l was below 15.7% for all DAPs. The revised approach proved to be feasible to routine assessment of occupational OP exposure and also for environmental background levels in the general population. In comparison with the previous approach, the revised version emphasizes the significance of adding pre-derivatization anhydration for higher sensitivity and precision

17 Product Authenticity

Haberli A, Girard P, Low MY, Ge XW*// *Hlth Sci Authority, Pharmaceut Lab, Applied Sci Grp, 11 Outram Rd, SG-169078 Singapore, Rep Singapore
J Pharm Biomed Anal 2010 **53** (1) 24
Isolation and structure elucidation of an interaction product of aminotadalafil found in an illegal health food product
An illegal health food product was found to contain an interaction product of aminotadalafil. To elucidate the structure of the interaction product, IR, NMR, and mass spectroscopy were employed. The previously unknown analyte was characterized as condensation product of aminotadalafil and hydroxymethylfuraldehyde. It probably results from a drug-excipient incompatibility

Lu YL, Zhou NL, Liao SY, Su N, He DX, Tian QQ, Chen B*, Yao SZ// *Hunan Normal Univ, MoE Key Lab Chem Biol & Traditional Chinese Med Res, CN-410081 Changsha, Peoples Rep China
Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2010 **27** (7) 893
Detection of adulteration of anti-hypertension dietary supplements and traditional Chinese medicines with synthetic drugs using LC/MS
Adulterants are sometimes found in dietary supplements and traditional Chinese medicines. These include drugs used for the treatment of anti-hypertension, including diuretics, calcium antagonists, and angiogenesis-converting enzyme inhibitors (ACEI). A sensitive and specific liquid chromatography-electrospray ionization mass spectrometry (LC/ESI-MS) approach has been developed for the analysis of 18 compounds. Separation was achieved on a Xtimate C18 reversed-phase column using a mixture of methanol, acetonitrile and 20 mM ammonium formate buffer (pH 3.2) as mobile phase. The technique displayed linearity from 0.03 to 21.52 mg/kg. Limits of detection ranged from 6.5 to 86.0 µg/kg. Recoveries from spiked samples ranged from 71% to 109%. The protocol was successfully employed in routine inspection analysis

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Anal Bioanal Chem 2010 **397** (5) 1927
Noninvasive detection of counterfeited ampoules of dexamethasone using NIR with confirmation by HPLC-DAD-MS and CE-UV methods
Counterfeit drug detection is routinely detected by means of near-infrared (NIR) measurements together with chemometric data processing. As might be expected, the most difficult counterfeits to detect are the "high quality fakes". These have the correct composition but are produced in violation of technological regulations by illegal manufacturers. An investigation was conducted into such forgeries and addresses important issues. Firstly was the application of NIR/chemometric to the detection of injectable formulations of drugs (in this case dexamethasone), i.e. aqueous solutions with low concentration of active ingredients and directly in the closed ampoules. Secondly was a comparison of NIR/chemometric conclusions with detailed chemical analysis

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Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2010 **27** (7) 903
Analysis of adulterated herbal medicines and dietary supplements marketed for weight loss by DOSY 1H-NMR
Diffusion ordered spectroscopy (DOSY) ^{1}H-nuclear magnetic resonance (NMR) and DOSY-COSY ^{1}H-NMR was employed to analyse 20 herbal medicines or dietary supplements marketed as natural slimming products. Analysis of whole samples and determination of both active and inactive ingredients in these complex matrices was achieved. Of the 20 formulations, 2 were entirely herbal and four contained ingredients declared on the packaging or the leaflet. However, the other 14 were adulterated. Eight formulations contained only sibutramine and at doses ranging from 4.4 to 30.5 mg/capsule. Five formulations contained sibutramine (from 5.0 to 19.6 mg/capsule or tablet) in combination with phenolphthalein (from 4.4 to 66.1 mg/capsule). The other formulation was adulterated with synephrine (19.5 mg/capsule). Quantification of the active components was performed with ^{1}H-NMR. Several other compounds were also identified including methylsynephrine, vitaberin, sugars and vitamins. Therefore, DOSY NMR is suggested as a practical approach for the detection of undeclared adulterants

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Sensor Actuator B Chem 2010 **147** (2) 392
Portable electronic nose based on carbon nanotube-SnO_2 gas sensors and its application for detection of methanol contamination in whiskeys
Hybrid carbon nanotube-SnO_2 gas sensors were employed as the basis of a portable electronic nose (E-nose). Electron beam (E-beam) evaporation with powder mixing was used to manufacture the hybrid gas sensors. The apparatus utilizes feature extraction techniques including integral and primary derivative. This results in higher classification performance as compared with the classical features (ΔR and $\Delta R/R_0$). Doping of carbon nanotube (CNT) was demonstrated to improve the sensitivity of hybrid gas sensors. The quantity of CNT was noted to have a direct effect on the selectivity of the volatile organic compounds, i.e., methanol (MeOH) and ethanol (EtOH). The E-nose was applied to authentic samples. The appartus is capable to both monitor and classify 1 vol% of MeOH content in whiskeys

18 Techniques

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Anal Chem 2010 **82** (9) 3454
Multiphoton ionization spectroscopy as a diagnostic technique of surfaces under ambient conditions
An important but challenging issue in analytical chemistry is the direct detection of solid substances. For the first time, laser multiphoton ionization spectroscopy has been employed for direct analysis of solids under ambient conditions. A solid powder/film was placed on a conductive surface and irradiated by a pulsed tunable laser whilst an electrical field of approximately 2 kV/cm was applied across this matrix and another electrode. Resulting photoelectrons and negative ions were measured by recording the current as a function of wavelength. This produced a multiphoton ionization spectrum that was characteristic of the surface. Rich spectral features were employed for identification of analytes. Sensitivity is in the low picomole range. This approach was successfully tested for direct detection of various organic molecules, including explosives, narcotic drugs, and polycyclic aromatic compound