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Current awareness in drug testing and analysis

1 Reviews

Barro R, Regueiro J, Llompart M, Garcia-Jares C*// *Univ Santiago de Compostela, Inst Invest & Anal Alimentarios, Dept Quim Anal, ES-15782 Santiago de Compostela, Spain
J Chromatogr A 2009 **1216** (3) 540
Analysis of industrial contaminants in indoor air: Part 1. Volatile organic compounds, carbonyl compounds, polycyclic aromatic hydrocarbons and polychlorinated biphenyls
Recent literature on the analysis of industrial contaminants in indoor air in the framework of the REACH project, which is mainly intended to improve protection of human health and the environment from the risks of more than 34 millions of chemical substances, is reviewed. Industrial pollutants which may occur in indoor air may be of very different types and origin, belonging to the volatile organic compounds (VOCs) and semivolatile organic compounds (SVOCs) categories. Several compounds have been classified into the priority organic pollutants (POPs) class for example, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and furans (PCDD/PCDFs) and related polychlorinated compounds, and polycyclic aromatic hydrocarbons (PAHs). Many of these compounds are partially to be found in the air gas phase, but also to the suspended particulate matter. In addition, settled dust can act as a concentrator for the less volatile pollutants and has become a matrix of great concern for indoors contamination. Analytical developments and applications regarding VOCs, aldehydes and other carbonyls, PCBs, PCDDs, PCDFs, and PAHs in the indoor environment reported during the last 10 years are reviewed. Sample collection and pretreatment, analyte extraction, clean-up procedures, determination techniques, performance results, as well as compound concentrations in indoor samples, are summarized and discussed.

Garcia-Jares C, Regueiro J, Barro R, Dagnac T, Llompart M// Address as above
J Chromatogr A 2009 **1216** (3) 567
Analysis of industrial contaminants in indoor air: Part 2. Emergent contaminants and pesticides
The literature on the analysis of several contaminants related to the industrial development in indoor air in the framework of the REACH project is reviewed. Attention is focused on emergent contaminants and biocides. Phthalates, polybrominated and phosphate flame retardants, fragrances, pesticides, as well as other emerging pollutants, are of increasing environmental and health concern and are extensively found in indoor air. Some are believed to act as priority organic pollutants (POPs) and/or endocrine disrupting compounds (EDC), and may be found both in air and associated to the suspended particulate matter (PM) and settled dust. Papers on analytical developments and applications regarding the considered contaminants in the indoor environment reported during the last 10 years are reviewed. Sample collection and pretreatment, analyte extraction or desorption, clean-up procedures, determination techniques, and performance results are summarized and discussed

Hansen SH// Univ Copenhagen, Fac Pharm, Dept Pharmaceut & Anal Chem, Universitetsparken 2, DK-2100 Copenhagen, Denmark
J Sep Sci 2009 **32** (5-6) 825
Sample preparation and separation techniques for bioanalysis of morphine and related substances
In present time the use or misuse of morphine and its derivatives are monitored by assaying the presence of the drug and its metabolites in biofluids. In the present review, focus is placed on the sample preparation and on the separation techniques used in the current best practices of bioanalysis of morphine and its major metabolites. However, as methods for testing the misuse of heroin, a morphine derivative, often involve bioanalytical methods that cover a number of other illicit drug substances, such methods are also included in the review. Furthermore, the review also includes bioanalysis in a broader perspective as analysis of plant materials, cell cultures and environmental samples. The review is not intended to cover all publications that include bioanalysis of morphine but is more to be considered a view into the current best practices of bioanalysis of morphine, its metabolites and other related substances

Maurer H// Univ Saarland, Inst Expt & Clin Pharmacol & Toxicol, Dept Expt & Clin Toxicol, DE-66421 Homburg, Germany
Anal Bioanal Chem 2009 **393** (1) 97
Mass spectrometric approaches in impaired driving toxicology
Similar to driving under the influence of alcohol, driving under the influence of prescribed or illegal drugs increases the risk of having road accidents. Therefore, an increasing number of blood samples must be analyzed for drugs on forensic toxicology. Immunoassays designed to detect a limited number of drugs (of abuse) may be applied as prescreening tests at the roadside and/or in the laboratory. However, many other common drugs, such as anesthetics, antidepressants, antiepileptics, antihistamines, newer designer drugs, herbal drugs, neuroleptics (antipsychotics), opioids, or sedative-hypnotics, may also impair drivers. This paper reviews multianalyte single-stage and tandem gas or liquid chromatography-mass spectrometry (GC-MS or LC-MS) techniques for the screening, identification, and validated quantification of such drugs in blood that have been noted since 2003. Basic information about the specimens, workup, chromatography, the mass spectral detection mode, and validation data are summarized in tables. The benefits and pitfalls of the procedures are critically discussed, particularly with respect to their probable applicability in impaired driving toxicology

Thevis M, Thomas A, Kohler M, Beuck S, Schanzer W// German Sport Univ Cologne, Inst Biochem, Ctr Preventive Doping Res, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany

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.

J Mass Spectrom 2009 **44** (4) 442

Special feature: Tutorial - Emerging drugs: Mechanism of action, mass spectrometry and doping control analysis

The number of compounds and doping methods in sports is in a state of constant flux. In addition to 'traditional' doping agents, such as anabolic androgenic steroids or erythropoietin, new therapeutics and emerging drugs have considerable potential for misuse in elite sport. Such compounds are commonly based on new chemical structures, and the mechanisms underlying their modes of action represent new therapeutic approaches arising from recent advances in medical research; therefore, sports drug testing procedures need to be continuously modified and complementary methods developed, preferably based on mass spectrometry, to enable comprehensive doping controls. This tutorial not only discusses emerging drugs that can be categorized as anabolic agents (selective androgen receptor modulators, SARMs), gene doping [hypoxia-inducible factor stabilizers, peroxisome-proliferator-activated receptor (PPAR)δ-agonists] and erythropoietin-mimetics (Hematide) but also compounds with potentially performance-enhancing properties that are not classified in the current list of the World Anti-Doping Agency. Compounds such as ryanodine-calstabin-complex modulators (benzothiazepines) are included, their mass spectrometric properties discussed, and current approaches in sports drug testing outlined

2 Sports Doping - General

Thevis M, Beuck S, Thomas A, Kortner B, Kohler M, Rodchenkov G, Schanzer W// German Sport Univ Cologne, Inst Biochem, Ctr Preventive Doping Res, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany

Rapid Commun Mass Spectrom 2009 **23** (8) 1139

Doping control analysis of emerging drugs in human plasma - Identification of GW501516, S-107, JTV-519, and S-40503

An important aspect of preventive doping research is the rapid implementation of tests for emerging drugs with potential for misuse into routine doping control assays. New therapeutics of different classes such as PPARδ-agonists (e.g. GW501516), ryanodine-calstabin-complex stabilizers (e.g. S-107 and JTV-519), and selective androgen receptor modulators (SARMs, e.g. S-40503) are currently used for the treatment of particular medical conditions such as metabolic syndrome, cardiac arrhythmia, debilitating diseases and osteoporosis, respectively. Due to their being at an early stage of clinical trials and the limited availability of data on the metabolism and possible renal elimination of the active drugs, the development of protocols for doping control analyses of plasma specimens could be an option for the detection of the circulating agents. The mass spectrometric fragmentation of four emerging drug candidates (GW501516, S-107, JTV-519, and S-40503) was elucidated by positive electrospray ionization and collision-induced dissociation using a high resolution/high accuracy mass spectrometer. A screening and confirmation procedure was established based on liquid chromatography/tandem mass spectrometry requiring a volume of 100 µl of plasma. Proteins were precipitated using acetonitrile, the specimens were centrifuged and the supernatant analyzed using a triple-quadrupole mass spectrometer employing multiple reaction monitoring of diagnostic ion transitions. The method was validated with regard to specificity, limits of detection (0.4-8.3 ng/ml), recoveries (72-98%), intraday and interday precisions (12-21%), and ion suppression/enhancement effects

Thevis M, Beuck S, Thomas A, Fussholler G, Sigmund G, Schlorer N, Rodchenkov G, Schafer M, Schanzer W// German Sport Univ Cologne, Inst Biochem, Ctr Preventive Doping Res, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany

Rapid Commun Mass Spectrom 2009 **23** (15) 2363

Electron ionization mass spectrometry of the ryanodine receptor-based Ca^{2+}-channel stabilizer S-107 and its implementation into routine doping control

New insights into the biochemistry of cardiac arrhythmia and skeletal muscle fatigue have yielded new drug candidates to counteract these phenomena. Major biological targets have become ryanodine receptor (RyR)-based Ca^{2+}-release channels, which tend to 'leak' under various circumstances including strenuous exercise and, thus, cause aberrant calcium sparks that entail impaired muscle function. Therapeutics, which are referred to as rycals, are currently being developed to treat cardiac arrhythmia *via* enhancement of calstabin-ryanodine affinities that causes a stabilization of the RyR. These therapeutics possess potential for misuse in sports, and an early implementation of target analytes such as the benzothiazepine derivatives S-107 and JTV-519 or putative metabolites into doping control screening procedures is recommended. Reference compounds, deuterated analogues, and a putative metabolic product were synthesized, and electron ionization mass spectra of these products were studied and dissociation pathways elucidated by means of tandem mass spectrometry (MS/MS) and accurate mass measurements. The characterized analytes were incorporated into existing sports drug testing assays based on liquid-liquid extraction and subsequent gas chromatography/mass spectrometry (GC/MS) analysis, and specificity, lower limit of detection (4-6 ng/ml), intraday and interday precision (1.5-17.2%), as well as recovery (63-66%) were determined. The established procedure proved suitable for routine doping control analysis to detect a potential misuse of the drug candidate S-107 in elite sport

Vahermo M, Suominen T, Leinonen A, Yli-Kauhaluloma J*// *Univ Helsinki, Fac Pharm, Div Pharmaceut Chem, POB 56, Viikinkaari 5 E, FI-00014 Helsinki, Finland

Arch Pharm (Weinheim) 2009 **342** (4) 201

Synthesis and characterization of hydroxylated mesocarb metabolites for doping control

The synthesis and method of analysis of hydroxylated mesocarb metabolites are described. Six potential hydroxylated mesocarb metabolites were prepared, characterized, and compared with the mesocarb metabolites synthesized enzymatically *in vitro* using human liver proteins and also compared with metabolites extracted from human urine after oral administration of mesocarb. *p*-Hydroxymesocarb was the most prevalent metabolite (conjugated and non-conjugated) observed. With respect to doping analysis, synthesis of *p*-hydroxymesocarb, the main urinary metabolite of mesocarb, and its availability as a reference material is important

3 Steroids

Biddle ST, O'Donnell A, Houghton E, Creaser C// Quotient Bioresearch Ltd, HFL Sport Science, Newmarket Rd, Fordham, Ely CB7 5WW, England

Rapid Commun Mass Spectrom 2009 **23** (5) 713

Metabolism of methyltestosterone in the greyhound

Gas chromatography/mass spectrometry and selective derivatisation techniques have been used to identify urinary metabolites of methyltestosterone following oral administration to the greyhound. Several metabolites were identified including reduced, mono-, di- and trihydroxylated steroids. The major metabolites observed were 17α-methyl-5β-androstane-3α-17β-diol, 17α-methyl-5β-androstane-3α,16α,17β-triol, and a further compound tentatively identified as 17α-methyl-5z-androstane-6z,17β-triol. The most abundant of these was the 17α-methyl-5β-androstane-3α,16α,17β-triol. This metabolite was identified by comparison with a reference standard synthesised using a Grignard procedure and characterised using trimethylsilyl (TMS) and acetonide-TMS derivatisation techniques. There did not appear to be any evidence for 16β-hydroxylation as a phase I metabolic transformation in the greyhound. However, significant quantities of 16α-hydroxy metabolites were detected. Selective enzymatic hydrolysis procedures indicated that the major metabolites identified were excreted as glucuronic acid conjugates. Metabolic transformations observed in the greyhound have been compared with those of other mammalian species and are discussed here

Galesio M, Rial-Otero R, Capelo-Martinez JL*// *Univ Nova Lisboa, Fac Ciencias & Technol, Dept Quim, ReQuimTe, PT-2829-516 Monte de Caparica, Portugal

Rapid Commun Mass Spectrom 2009 **23** (12) 1783

Comparative study of matrices for their use in the rapid screening of anabolic steroids by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry

New data on sample preparation and matrix selection for the fast screening of androgenic anabolic steroids (AAS) by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) is presented. The rapid screening of 15 steroids included in the World Anti-Doping Agency (WADA) prohibited list using MALDI was evaluated. Nine organic and two inorganic matrices were assessed in order to determine the best matrix for steroid identification in terms of ionisation yield and interference by characteristic matrix ions. The best results were achieved for the organic matrices 2-(4-hydroxyphenylazo)benzoic acid (HABA) and *trans*-3-indoleacrylic acid (IAA). Good signals for all the steroids studied were obtained for concentrations as low as 0.010 and 0.050 µg/ml on the MALDI sample plate for the HABA and IAA matrices, respectively. For these two matrices, the sensitivity achieved by MALDI is comparable with the sensitivity achieved by gas chromatography/mass spectrometry (GC/MS), which is the conventional technique used for AAS detection. Furthermore, the accuracy and precision obtained with MALDI are very good, since an internal mass calibration is performed with the matrix ions. For the inorganic matrices, laser fluences higher than those used with organic matrices are required to obtain good MALDI signals. When inorganic matrices were used in combination with glycerol as a dispersing agent, an important reduction of the background noise was observed. Urine samples spiked with the study compounds were processed by solid-phase extraction (SPE) and the screening was consistently positive

Parr MK, Fussholler G, Schlorer N, Opfermann G, Piper T, Rodchenkov G, Schanzer W// German Sport Univ Cologne, Inst Biochem, Ctr Preventive Doping Res, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany

Rapid Commun Mass Spectrom 2009 **23** (2) 207

Metabolism of androsta-1,4,6-triene-3,17-dione and detection by gas chromatography/mass spectrometry in doping control

The urinary metabolism of the irreversible aromatase inhibitor androsta-1,4,6-triene-3,17-dione was investigated. It is mainly excreted unchanged and as its 17β-hydroxy analogue. For confirmation, 17β-hydroxyandrosta-1,4,6-trien-3-one was synthesized and characterized by nuclear magnetic resonance (NMR) in addition to the parent compound. In addition, several reduced metabolites were detected in the post-administration urines, namely 17β-hydroxyandrosta-1,4-dien-3-one (boldenone), 17β-hydroxy-5β-androst-1-en-3-one (boldenone metabolite), 17β-hydroxyandrosta-4,6-dien-3-one, and androsta-4,6-diene-3,17-dione. The identification was performed by comparison of the metabolites with reference material utilizing gas chromatography/mass spectrometry (GC/MS) of the underivatized compounds and GC/MS and GC/tandem mass spectrometry (MS/MS) of their trimethylsilyl (TMS) derivatives. Alterations in the steroid profile were also observed, most obviously in the androsterone/testosterone ratio. Even if not explicitly listed, androsta-1,4,6-triene-3,17-dione is classified as a prohibited substance in sports by the World Anti-Doping Agency (WADA) due to its aromatase-inhibiting properties. In 2006 three samples from human routine sports doping control tested positive for metabolites of androsta-1,4,6-triene-3,17-dione. The samples were initially found suspicious for the boldenone metabolite 17β-hydroxy-5β-androst-1-en-3-one. Since metabolites of androst-4-ene-3,6,17-trione were also present in the urine samples, it is presumed that these findings were due to the administration of a product like 'Novedex Xtreme', which could be easily obtained from the sport supplement market

Peters RJB, Van Engelen MC, Touber ME, Georgakopoulus C, Nielen MWF// Inst Food Safety, PO Box 230, NL-6700 AE Wageningen, The Netherlands

Rapid Commun Mass Spectrom 2009 **23** (15) 2329

Searching for *in silico* predicted metabolites and designer modifications of (cortico)steroids in urine by high-resolution liquid chromatography/time-of-flight mass spectrometry

Glucocorticosteroids are a restricted class of substances and appear on the 'in-competition' prohibited list of the World Anti-Doping Agency (WADA). Analysis of glucocorticosteroids is complicated since they show significant phase 1 and 2 metabolism in the human body and are excreted into urine in concentrations in the μg/l range. Full scan, high-resolution time-of-flight mass spectrometry analysis generates information on all ionisable components in urine, including known and unknown metabolites of steroids and even designer modifications of anabolic steroids. However, evaluation of the data obtained can be difficult and time-consuming because of the need to differentiate between endogenous components and compounds of interest. MetaboLynx, a spectral and chromatographic search program, was modified for the determination of *in silico* predicted metabolites of glucocorticosteroids and designer modifications of anabolic steroids in human urine. Spiked urine samples were successfully screened for known components in a targeted approach and for unknown species in a non-targeted approach using data filtering to limit potential false-positives. A simplified combined approach of targeted and untargeted screening was used for the detection of metabolites and designer modifications of existing compounds. This approach proved successful and showed its strength in the detection of tetrahydrogestrinone (THG), a designer modification of gestrinone. THG was positively detected in a spiked urine sample and correctly identified as a twofold hydrogenation of gestrinone. The developed screening method can easily be adapted to specific needs and it is envisaged that a similar approach would be amendable to the discovery of metabolites or designer modifications of other compounds of interest

Piper T, Thevis M, Flenker U, Schanzer W// German Sport Univ Cologne, Inst Biochem, Ctr Preventive Doping Res, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany

Rapid Commun Mass Spectrom 2009 **23** (13) 1917

Determination of the deuterium/hydrogen ratio of endogenous urinary steroids for doping control purposes

The development and application of a combined gas chromatography/thermal conversion/isotope ratio mass spectrometry (GC/TC/IRMS) method for D/H ratio determination of endogenous urinary steroids are presented. The key element in sample preparation was the consecutive cleanup with high-performance liquid chromatography of initially native and subsequently acetylated steroids. This strategy enabled sufficient cleanup off all target analytes for determination of their respective D/H values. Ten steroids (11β-hydroxyandrosterone, 5α-androst-16-en-3α-ol, pregnanediol, androsterone, etiocholanolone, testosterone, epitestosterone, 5α-androstan-3α,17β-diol, 5β-androstan-3α,17β-diol and dehydroepiandrosterone) were measured from a single urine specimen. Depending on the biological background, the determination limit for all steroids ranged from 10 to 15 ng/ml for a 20 ml specimen. The method was validated by application of linear mixing models on each steroid and covered repeatability and reproducibility. The specificity of the procedure was ensured by gas chromatography/mass spectrometry (GC/MS) analysis of the sample using equivalent chromatographic conditions to those employed in the GC/TC/IRMS measurement. Within the sample preparation, no isotopic fractionation was observed, and no amount-dependent shift of the D/H ratios during the measurement was noticed. Possible memory effects occurring during IRMS measurements were corrected by applying a simple rule of proportion. In order to determine the naturally occurring D/H ratios of all implemented steroids, a population of 18 male subjects was analyzed. Relevant mean δ values among selected steroids were calculated which allowed us to study the metabolic pathways and production sites of all the implemented steroids with additional consideration of the corresponding $^{13}C/^{12}C$ ratios

Shen M, Xiang P, Shen BH, Bu J, Wang MY// Ministry Justice, Inst Forensic Sci, Dept Forensic Toxicol, Shanghai Key Lab Forensic Med, CN-200063 Shanghai, Peoples Rep China

Forensic Sci Int 2009 **184** (1-3) 32

Physiological concentrations of anabolic steroids in human hair

The analysis of anabolic steroid levels in human hair is required in order to distinguish between pharmaceutical steroids and natural steroids. The simultaneous identification and quantitation of five endogenous anabolic steroids (testosterone, epitestosterone, androsterone, etiocholanolone and dehydroepiandrosterone) in hair was achieved using gas chromatography-tandem mass spectrometry (GC/MS/MS). Following basic hydrolysis, hair samples were extracted with diethyl ether, derivatized and then detected using GC/MS/MS in the multiple-reaction monitoring mode (MRM). The one precursor/two product ion transitions for each anabolic steroid were monitored. The limits of detection for the five endogenous anabolic steroids were in the 0.1-0.2 pg/mg range. All analytes showed good linearity and the extraction recoveries were 74.6-104.5%. Within-day and between-day precisions were less than 20%. Testosterone, epitestosterone, androsterone, etiocholanolone, and dehydroepiandrosterone in human hair were analysed. Full-length hair samples were taken at the skin surface from the vertex of 39 males, 30 females and 11 children from China none of which were professional athletes. Testosterone and dehydroepiandrosterone were identified in all the hair segments. The physiological concentrations of testosterone were in the range 0.8-24.2 pg/mg, 0.1-16.8 pg/mg and 0.2-11.5 pg/mg in males, females and children, respectively. However, the mean values of dehydroepiandrosterone were much higher than the concentrations of testosterone. These results provide reference values which may form the basis for the interpretation of results from investigations into the abuse of endogenous anabolic steroids

Zhang L, Chen JF, He Y, Chi YW, Chen GN*// *Fuzhou Univ, MoE Key Lab Anal & Detect Food Safety, CN-350002 Fuzhou, Fujian, Peoples Rep China

Talanta 2009 **77** (3) 1002

A new mixed micellar electrokinetic chromatography method for analysis of natural and synthetic anabolic steroids

The analysis of five neutral anabolic steroids has been achieved using a simple, rapid and low-costing new mixed surfactant MEKC method. Bile salt coupling with Triton X-100 was a suitable bi-micellar surfactant for the separation of these anabolic steroids with similar structure. Five natural and synthetic anabolic steroids, such as androstenedione (AD), 19-norandrostenedione (NAD), 1,4-androstadiene-3,17-dione (ADD), methandrostenolone (MA) and methyltestosterone (MT) were separated and detected in an alkaline buffer system (pH 9.0) containing 15 mM Britton-Robinson (BR) buffer, 50mM sodium cholate (SC) and 0.1% (v/v) Triton X-100 with detection wavelength at 241 nm and 18 kV of separation voltage. Five coexistent neutral steroids were completely separated within 12 min with the detection limits ranged from 0.20 to 0.51 μg/ml. The technique was successfully used for detection and confirmation of the anabolic steroid methandrostenolone in methandrostenolone tablets and in the real human urine. GC-MS was employed to confirm the free methandrostenolone existence in the urine sample in order to validate the reliability of MEKC method

4 Peptides

Thomas A, Kohler M, Schanzer W, Kamber M, Delahaut P, Thevis M// German Sport Univ Cologne, Inst Biochem, Ctr Preventive Doping Res, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany

Rapid Commun Mass Spectrom 2009 **23** (17) 2669

Determination of Synacthen in urine for sports drug testing by means of nano-ultra-performance liquid chromatography/tandem mass spectrometry

Doping control analysis of performance-enhancing peptides in urine represents a challenging requirement in modern sports drug testing. Low dosing, effective metabolism and short half-life lead to target concentrations in the low fmol/ml range in urine. Synthetic adrenocorticotropic hormone (1-24, Syn-ACTH-en) shares all these characteristics and improved analytical performance is required for its sufficient determination by means of liquid chromatography/tandem mass spectrometry (LC/MS/MS). The desired effects for cheating sportsmen are mainly due to enhanced release of corticosteroids as well as androgenic steroids into the circulation after systemic administration of the drug. Immunoaffinity purification with coated magnetic beads and subsequent liquid chromatography with nano-ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (high resolution/high mass accuracy) of Synacthen from urinary specimens is described in the present study. The general proof of principle was obtained by analysis of excretion study urine samples and validation was performed with focus on the limit of detection (3 pg/ml), linearity, precision (<20%), recovery (approximately 30%), robustness, specificity and stability. For all experiments, the ACTH fragment 1-17 was used as the internal standard

6 CNS Agents

Lu J, Wang X, Yang S, Liu X, Qin Y, Shen L, Wu Y, Xu Y, Wu M, Ouyang G*// *Sun Yat-Sen Univ, Sch Chem & Chem Engn, CN-510275 Guangzhou, Peoples Rep China

Rapid Commun Mass Spectrom 2009 **23** (11) 1592

Doping control analysis for adrafinil and its major metabolites in human urine

A new and reliable two-step liquid chromatography/tandem mass spectrometry (LC/MS/MS) method in combination with gas chromatography/mass spectrometry (GC/MS) for the screening and confirmation of adrafinil and its major metabolites, modafinil and modafinil acid, in human urine has been developed and validated. The method involved reversed-phase C_{18} solid-phase extraction (SPE) cartridge extraction and MS analysis by means of LC/MS/MS and GC/MS. The study illustrated that the ESI capillary temperature played a key role in the formation of the protonated molecule. The limits of detection (LODs) of the developed method for the three compounds were lower than the minimum required performance limit (MRPL) of the World Anti-Doping Agency (WADA). The human urine samples obtained after the oral administration of modafinil and from the Beijing 2008 Olympic Games were analyzed by using the described method, which has also been successfully applied to routine analyses and the WADA Proficiency Test

Montes de Oca Porto R, Rosado Perez A, Correa Vidal MT, Granda Fraga M// Inst Med Deporte, Lab Antidoping, Calle 100 y Aldabo, CU-10800 Ciudad de la Habana, Cuba

Rapid Commun Mass Spectrom 2009 **23** (2) 249

Qualitative confirmation procedure for ephedrines as acetonide derivatives in doping urine samples by gas chromatography/electron ionization mass spectrometry

Ephedrines are sympathomimetic amines which have central nervous system stimulating properties and, for this reason, some of them are forbidden in sport by the World Antidoping Agency (WADA). They are screened and quantitated in urine by several published techniques and confirmed by gas chromatography/mass spectrometry (GC/MS). In this paper, a simple and easy confirmation procedure for norpseudoephedrine, norephedrine, ephedrine and pseudoephedrine in human urine by GC/electron ionization (EI)-MS is described. After the addition of diphenylamine as internal standard, a liquid-liquid extraction procedure under alkaline conditions with *tert*-butyl methyl ether was applied to the samples. The analytes were derivatized with acetone and pyridine to form the correspondent oxazolidine derivatives (acetonide). The EI mass spectra of all the studied substances have many diagnostic ions with relative abundance in accordance with WADA requirements and show great structural information content. The fragmentation of theses derivatives is discussed

7 Equine

Barton C, Beck P, Kay R, Teale P, Roberts J// Quotient Bioresearch Ltd, HFL Sport Science, Newmarket Rd, Fordham CB7 5WW, England

Proteomics 2009 **9** (11) 3058

Multiplexed LC-MS/MS analysis of horse plasma proteins to study doping in sport

The development of protein biomarkers for the indirect detection of doping in horse is a potential solution to doping threats such as gene and protein doping. A method for biomarker candidate discovery in horse plasma is presented using targeted analysis of proteotypic peptides from horse proteins. These peptides were first identified in a novel list of the abundant proteins in horse plasma. To monitor these peptides, an LC-MS/MS method using multiple reaction monitoring was developed to study the quantity of 49 proteins in horse plasma in a single run. The method was optimised and validated, and then applied to a population of race-horses to study protein variance within a population. The method was finally applied to longitudinal time courses of horse plasma collected after administration of an anabolic steroid to demonstrate utility for hypothesis-driven discovery of doping biomarker candidates

De Leo M, Giorgi M, Saccomanni G, Manera C, Braca A// Univ Pisa, Dipt Chim Bioorgan & Biofarm, Via Bonanno 33, IT-56126 Pisa, Italy

Rapid Commun Mass Spectrom 2009 **23** (2) 228

Evaluation of tramadol and its main metabolites in horse plasma by high-performance liquid chromatography/fluorescence and liquid chromatography/electrospray ionization tandem mass spectrometry techniques

Tramadol is a centrally acting analgesic drug that has been used clinically for the last two decades to treat pain in humans. The clinical response of tramadol is strictly correlated to its metabolism, because of the different analgesic activity of its metabolites. *O*-Desmethyltramadol (M1), its major active metabolite, is 200 times more potent at the μ-receptor than the parent drug. In recent years tramadol has been widely introduced in veterinary medicine but its use has been questioned in some species. The aim of the present study was to develop a new sensible method to detect the whole metabolic profile of the drug in horses, through plasma analyses by high-performance liquid chromatography (HPLC) coupled with fluorimetric (FL) and photodiode array electrospray ionization mass spectrometric (PDA-ESI-MS) detection, after its sustained release by oral administration (5 mg/kg). In HPLC/FL experiments the comparison of the horse plasma chromatogram profile with that of a standard mixture suggested the identification of the major peaks as tramadol and its metabolites M1 and *N*,*O*-desmethyltramadol (M5). LC/PDA-ESI-MS/MS analysis confirmed the results obtained by HPLC/FL and also provided the identification of two more metabolites, *N*-desmethyltramadol (M2), and *N*,*N*-didesmethyltramadol (M3). Another metabolite, M6, was also detected and identified. The present findings demonstrate the usefulness and the advantage of LC/ESI-MS/MS techniques in a search for tramadol metabolites in horse plasma samples

Dirikolu L, Woods WE, Boyles J, Lehner AF, Harkins JD, Fisher M, Schaeffer DJ, Tobin T// Univ Illinois, Coll Veterinary Med, Dept Veterinary Biosci, 3830 VMBSB, 2001 Sth Lincoln Ave, Urbana, Il 61802, USA

J Vet Pharmacol Ther 2009 **32** (3) 271

Nonsteroidal anti-inflammatory agents and musculoskeletal injuries in thoroughbred racehorses in Kentucky

Injuries sustained by horses during racing have been considered as an unavoidable part of horse racing. Many factors may be associated with the musculoskeletal injuries of Thoroughbred race horses. This study surveyed the amounts of nonsteroidal anti-inflammatory agents (NSAIDs) in injured horse's biological system (plasma) at Kentucky racetracks from January 1, 1995 through December 31, 1996. During that period, there were 84 catastrophic cases (euthanized horses) and 126 noncatastrophic cases. Plasma concentrations of NSAIDs were determined by high performance liquid chromatography in injured and control horses. The possible role of anti-inflammatory agents in musculoskeletal injuries of Thoroughbred race horses was investigated by comparing the apparent concentrations of NSAIDs in injured horses to concentrations in control horses. The plasma concentrations of phenylbutazone and flunixin were higher in injured horses than in control horses. Most injured and control horses did not have a detectable level of naproxen in their plasma samples. Further studies must be carried out to determine whether horses with higher plasma concentrations of NSAIDs have an altered risk of musculoskeletal injuries compared with other horses

Fidani M, Gamberini MC, Pasello E, Palazzoli F, De Iuliis P, Montana M, Arioli F*// *Univ Milan, Dept Veterinary Sci & Technol Food Safety, Via Celoria 10, IT-20133 Milan, Italy

Rapid Commun Mass Spectrom 2009 **23** (1) 65

Evaluation of equine urine reactivity towards phase II metabolites of 17-hydroxy steroids by liquid chromatography/tandem mass spectrometry

Proper storage conditions of biological samples are fundamental to avoid microbiological contamination that can cause chemical modifications of the target analytes. A simple liquid chromatography/tandem mass spectrometry (LC/MS/MS) method through direct injection of diluted samples, without prior extraction, was used to evaluate the stability of phase II metabolites of boldenone and testosterone (glucuronides and sulphates) in intentionally poorly stored equine urine samples. We also considered the stability of some deuterated conjugated steroids generally used as internal standards, such as deuterated testosterone and epitestosterone glucuronides, and deuterated boldenone and testosterone sulphates. The urines were kept for 1 day at room temperature, to mimic poor storage conditions, then spiked with the above steroids and kept at different temperatures (-18°C, 4°C, room temperature). It has been possible to confirm the instability of glucuronide compounds when added to poorly stored equine urine samples. In particular, both 17β- and 17α-glucuronide steroids

were exposed to hydrolysis leading to non-conjugated steroids. Only 17β-hydroxy steroids were exposed to oxidation to their keto derivatives whereas the 17α-hydroxy steroids were highly stable. The sulphate compounds were completely stable. The deuterated compounds underwent the same behaviour as the unlabelled compounds. The transformations were observed in urine samples kept at room temperature and at a temperature of 4°C (at a slower rate). No modifications were observed in frozen urine samples. In the light of the latter results, the immediate freezing at -18°C of the collected samples and their instant analysis after thawing is the proposed procedure for preventing the transformations that occur in urine, usually due to microbiological contamination

Tevell Aberg A, Olsson C, Bondesson U, Hedeland M*// *Nat Veterinary Inst, SVA, Dept Chem, SE-75189 Uppsala, Sweden
J Mass Spectrom 2009 **44** (7) 1026
A mass spectrometric study on meloxicam metabolism in horses and the fungus *Cunninghamella elegans*, and the relevance of this microbial system as a model of drug metabolism in the horse
This paper describes a study where the metabolism of the non-steroidal anti-inflammatory drug meloxicam was investigated in six horses and in the filamentous fungus *Cunninghamella elegans*. The metabolites identified were compared between the species, and then the fungus was used to produce larger amounts of the metabolites for future use as reference material. *C. elegans* proved to be a good model of phase I meloxicam metabolism in horses since all four metabolites found were the same in both species. Apart from the two main metabolites, 5'-hydroxymethylmeloxicam and 5'-carboxymeloxicam, a second isomer of hydroxymeloxicam and dihydroxylated meloxicam were detected for the first time in horse urine and the microbial incubations. Phase II metabolites were not discovered in the *C. elegans* samples but hydroxymeloxicam glucuronide was detected intact in horse urine for the first time in this study. Urine from six horses was further analyzed in a semi-quantitative sense and 5'-hydroxymethylmeloxicam gave peaks with much higher intensity compared to the parent drug and the other metabolites, and was detected for at least 14 days after the last given dose in some of the horses. From the results presented in this article, we suggest that analytical methods developed for the detection of meloxicam in horse urine after prohibited use should focus on the 5'-hydroxymethyl metabolite and that *C. elegans* can be used to produce large amounts of this metabolite for potential future use as a reference compound

Vonaparti A, Lyris E, Panderi I, Koupparis M, Georgakaopoulos C*// *Olympic Athletic Ctr Athens Spiros Louis, Doping Control Lab Athens, 37 Kifissias Ave, GR-15123 Maroussi, Greece
Rapid Commun Mass Spectrom 2009 **23** (7) 1020
Direct injection liquid chromatography/electrospray ionization mass spectrometric horse urine analysis for the quantification and confirmation of threshold substances for doping control. II. Determination of theobromine
In equine sport, theobromine is prohibited with a threshold level of 2 μg/ml in urine, hence doping control laboratories have to establish quantitative and qualitative methods for its determination. Two simple liquid chromatography/mass spectrometry (LC/MS) methods for the identification and quantification of theobromine were developed and validated using the same sample preparation procedure but different mass spectrometric systems: ion trap mass spectrometry (ITMS) and time-of-flight mass spectrometry (TOFMS). Particle-free diluted urine samples were directly injected into the LC/MS systems, avoiding the time-consuming extraction step. 3-Propylxanthine was used as the internal standard. The tested linear range was 0.75-15 μg/ml. Matrix effects were evaluated analyzing calibration curves in water and different fortified horse urine samples. A great variation in the signal of theobromine and the internal standard was observed in different matrices. To overcome matrix effects, a standard additions calibration method was applied. The relative standard deviations of intra- and inter-day analysis were lower than 8.6 and 7.2%, respectively, for the LC/ITMS method and lower than 5.7 and 5.8%, respectively, for the LC/TOFMS method. The bias was less than 8.7% for both methods. The methods were applied to two case samples, demonstrating simplicity, accuracy and selectivity

You Y, Uboh CE*, Soma LR, Guan F, Li X, Rudy JA, Liu Y, Chen J// *West Chester Univ, Dept Chem, Equine Toxicol & Res Ctr, 220 East Rosedale Ave, West Chester, Pa 19382, USA
Rapid Commun Mass Spectrom 2009 **23** (13) 2035
Ultra-performance liquid chromatography/tandem mass spectrometry in high-throughput detection, quantification and confirmation of anabolic steroids in equine plasma
An ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) method for fast-throughput analysis of eight anabolic and androgenic steroids (AAS) in equine plasma is reported. Analytes were recovered by liquid-liquid extraction using methyl *tert*-butyl ether, separated on a 1.9 μm C_{18} reversed-phase column, and analyzed in positive electrospray ionization mode on a triple quadrupole mass spectrometer with selected reaction monitoring (SRM) and full product ion scans. Two SRM ion transitions were monitored for each AAS during screening to obtain highly selective screening results. Full product ion spectra of excellent quality for AAS, at 100 pg/0.5 ml in plasma, devoid of interfering spectra from impurities in plasma, were obtained. To our knowledge, this is the first report on the acquisition of full product ion spectra at such a low analyte concentration and plasma volume using a triple quadrupole instrument. In addition to product ion intensity ratios obtained from three SRM scans for identifying AAS in equine plasma, full product ion spectra were used as supporting evidence for confirmation. For quantification, deuterium-labeled testosterone and stanozolol were used as internal standards (ISs). The limits of detection, quantification and confirmation were 6.25-12.5 pg/0.5 ml, 25 pg/0.5 ml and 50-100 pg/0.5 ml, respectively. There was no significant matrix effect on the analysis of all eight AAS. Intra-day precision and accuracy were 2-15% and 91-107%, respectively. Inter-day precision and accuracy were 1-21% and 94-110%, respectively. Total analysis time was 5 min. To date, the method has been successfully used in the analysis of >12,000 samples for AAS in plasma from racehorses competing in the State of Pennsylvania. The method is fast, selective, reproducible, and reliable

8 Recreational Drugs - General

Chen CY, Shen CC, Yang TJ, Chang YZ, Lee MR*// *Natl Chung Hsing Univ, Dept Chem, 250 Kuo Kung Rd, Taiching 40227, Taiwan
Talanta 2009 **77** (4) 1351
High throughput screening various abused drugs and metabolites in urine by liquid chromatography-heated electrospray ionization/tandem mass spectrometry
High throughput screening of various abused drugs in urine by liquid chromatography-heated electrospray ionization/tandem mass spectrometry was evaluated. Chromatographic analysis was achieved using a C_{18} reverse phase column using a linear gradient of 10mM ammonium acetate containing 0.1% formic acid-methanol as mobile phase and the total separation time was 7 min. Simple and rapid sample preparation was achieved by passing urine samples through a 0.22 μm PVDF syringe filter. The detection limits of the studied abused drugs in urine were from 0.6 ng/ml (ketamine) to 9.0 ng/ml (norcodeine). The linear range was from 1 to 1200 ng/ml with relative standard deviation (R.S.D.s) value below 14.8% (intra-day) and 24.6% (inter-day). Urine samples from drug-abuse suspects and ketamines and amphetamines were detected in suspect samples. The results illustrate the applicability of LC-HESI-MS/MS for high throughput screening of the various abused drugs in urine

Chen LC, Hashimoto Y, Furuya H, Takekawa K, Kubota T, Hiraoka K*// *Univ Yamanashi, Clean Energy Res Ctr, 4-3-11 Takeda, Kofu, Yamanashi 400 8511, Japan
Rapid Commun Mass Spectrom 2009 **23** (3) 333
Rapid detection of drugs in biofluids using atmospheric pressure chemi/chemical ionization mass spectrometry
We have demonstrated that, with simple pH adjustment, volatile drugs such as methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), ketamine, and valproic acid could be analyzed rapidly from raw biofluid samples (e.g. urine and serum) without dilution, or extraction, using atmospheric pressure ionization. The ion source was a variant type of atmospheric pressure chemical ionization (APCI) that used a dielectric barrier discharge (DBD) to generate the metastable helium gas and reagent ions. The sample solution was loaded in a disposable glass pipette, and the volatile compounds were purged by nitrogen gas to be reacted with the metastable helium gas. The electrodes of the DBD were arranged in such a way that the generated glow discharge was confined within the discharge tube and was not exposed to the analytes. A needle held at 100-500 V was placed between the ion-sampling orifice and the discharge tube to guide the analyte ions into the mass spectrometer. After pH adjustment of the biofluid sample, the amphiphilic drugs were in the form of a water-insoluble oil, which could be concentrated on the liquid surface. By gentle heating of the sample to increase the evaporation rate, rapid and sensitive detection of these drugs in raw urine and serum samples could be achieved in less than 2 min for each sample

Luosujarvi L, Laakkonen UM, Kostiainen R, Kotiaho T, Kauppila TJ*// *Univ Helsinki, Fac Pharm, Div Pharmaceut Chem, PO Box 56, FI-00014 Helsinki, Finland
Rapid Commun Mass Spectrom 2009 **23** (9) 1401
Analysis of street market confiscated drugs by desorption atmospheric pressure photoionization and desorption electrospray ionization coupled with mass spectrometry
No abstract available

Sauer C, Peters FT, Haas C, Meyer MR, Fritschi G, Maurer HH*// *Saarland Univ, Dept Experiment & Clin Toxicol, DE-66421 Homburg, Germany

J Mass Spectrom 2009 **44** (6) 952

New designer drug -pyrrolidinovalerophenone (PVP): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques

The aim of the present study was to identify the metabolites of the new designer drug α-pyrrolidinovalerophenone (PVP) in rat urine using GC/MS techniques. Eleven metabolites of PVP could be identified suggesting the following metabolic steps: hydroxylation of the side chain followed by dehydrogenation to the corresponding ketone; hydroxylation of the 2"-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam or followed by ring opening to the respective aliphatic aldehyde and further oxidation to the respective carboxylic acid; degradation of the pyrrolidine ring to the corresponding primary amine; and hydroxylation of the phenyl ring, most probably in the 4'-position. The authors' screening procedure for pyrrolidinophenones allowed the detection of PVP metabolites after application of a dose corresponding to a presumed user's dose. In addition, the involvement of nine different human cytochrome P450 (CYP) isoenzymes in the side chain hydroxylation of PVP was investigated and CYP 2B6, 2C19, 2D6, and 3A4 were found to catalyze this reaction

Sergi M, Bafile E, Compagnone D, Curini R, D'Ascenzo G, Romolo FS// Univ Teramo, Dept Food Sci, Via CR Lerici 1, IT-64023 Mosciano SA, Teramo, Italy

Anal Bioanal Chem 2009 **393** (2) 709

Multiclass analysis of illicit drugs in plasma and oral fluids by LC-MS/MS

A technique for the simultaneous determination of several illicit drugs belonging to different chemical and toxicological classes in human plasma and oral fluids is described. Amphetamine, methamphetamine, morphine, 6-monoacetylmorphine, methylenedioxyamphetamine, methylenedioxyethylamphetamine, methylenedioxymethamphetamine, cocaine, benzoylecgonine, tetrahydrocannabinol, carboxytetrahydrocannabinol, ketamine, and phencyclidine have been quantified in real samples using a very rapid sample treatment which is basically a protein precipitation. Quantitative analysis was performed by liquid chromatography-tandem mass spectrometry and has been fully validated. All the analytes were detected in positive ionization mode using a TurboIonSpray source, except carboxytetrahydrocannabinol, which was detected in negative ionization mode. The use of a diverter valve between the column and the mass spectrometer facilitated the preservation of the ion source performances for high-throughput analysis

Simoes SS, Ajenjo AC, Franco JM, Vieira DN, Dias MJ// Nat Inst Legal Med-South Branch, Rua Manuel Bento de Sousa 3, PT-1150-219 Lisbon, Portugal

Rapid Commun Mass Spectrom 2009 **23** (10) 1451

Liquid chromatography/tandem mass spectrometry for the qualitative and quantitative analysis of illicit drugs and medicines in preserved oral fluid

A qualitative and quantitative analytical method was developed for the simultaneous determination of 24 illicit drugs and medicines, in preserved oral fluid samples collected with the StatSure Saliva Sampler collection device. The samples were prepared by liquid-liquid extraction followed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis. The chromatographic separation was performed with an Atlantis T3 (100 x 2.1 mm i.d., 3 µm) reversed-phase column using an acetonitrile/2 mM ammonium formate buffer pH 3.4 gradient and the MS/MS detection was achieved with two precursor-product ion transitions per substance. The method was fully validated, including specificity and capacity of identification, limit of detection (0.2-2.1 µg/l), limit of quantitation (0.8-6.4 µg/l), recovery (34-98%), carryover, linearity (the method was linear in the range 1-200 µg/l), intra-assay precision (coefficient of variance (CV) <20% for 20 µg/l and CV % for 100 µg/l) and inter-assay accuracy (mean relative error <15%) and precision (CV <20%). The method showed to be specific and sensitive. It has already been successfully used in four proficiency tests and subsequently applied to oral fluid samples collected from road traffic volunteers in the driving population of Portugal (districts of Lisbon, Coimbra and Porto), within the DRUID project

Takahashi M, Nagashima M, Suzuki J, Seto T, Yasuda I, Yoshida T// Tokyo Metropolitan Inst Publ Hlth, 3-24-1 Hyakunincho, Shinjyuku ku, Tokyo 169 0073, Japan

Talanta 2009 **77** (4) 1245

Creation and application of psychoactive designer drugs data library using liquid chromatography with photodiode array spectrophotometry detector and gas chromatography-mass spectrometry

To rapidly identify a potentially hazardous psychoactive designer drug (a compound in which part of the molecular structure of a stimulant or narcotic has been modified), a psychoactive drugs data library was created by performing analysis using liquid chromatography with photodiode array spectrophotometry (LC/PDA) and gas chromatography-mass spectrometry (GC/MS). The data icomprise of the LC capacity factor (*k'*) ratios in relation to the internal standard, the ultraviolet (UV) spectra and the MS spectra of 104 compounds. A comparative study of the data in this report with the analytical data for commercial and illegal drug products quickly identified the psychoactive designer drugs in 205 purchased products by using the library. In addition, it was possible to analogize the structure of drugs for which there is no matching data in the library using similar data. Structural isomers of controlled substances detected from the library may predict their biological effects on humans, thus facilitating their public regulation. Examples include the narcotic 3,4,5-trimethoxyamphetamine (TMA) and its positional isomers 2,4,5-trimethoxyamphetamine (TMA-2) and 2,4,6-trimethoxyamphetamine (TMA-6), or the narcotic 1-(3-chlorophenyl)piperazine (3CPP) and its isomers 1-(*o*-chlorophenyl)piperazine (2CPP) and 1-(*p*-chlorophenyl)piperazine (4CPP).

West MJ, Went MJ*// *Univ Kent, Sch Phys Sci, Canterbury CT2 7NH, England

Spectrochim Acta A Mol Biol Spectrosc 2009 **71** (5) 1984

The spectroscopic detection of drugs of abuse in fingerprints after development with powders and recovery with adhesive lifters

An effective and reliable method for developing latent fingerprints is the long established method of application of powders. Fingerprints developed in situ at a crime scene routinely undergo lifting with specialist tapes. They are then subsequently stored in evidence bags which facilitates secure transit and preserves the chain of evidence. Previously, it has shown that exogenous material within a fingerprint may be detected using Raman spectroscopy following development with powders and lifting with adhesive tapes. The present study involved the application of Raman spectroscopy for the analysis of drugs of abuse in fingerprints that had been treated with powders and also subsequently lifted with adhesive tapes. Contaminated fingerprints were deposited on clean glass slides. Aluminium or iron based powders applied to contaminated fingerprints did not interfere with the Raman spectra obtained for the contaminants. Contaminated fingerprints developed with powders and then lifted with lifting tapes were also examined. The combination of these two techniques did not interfere with the successful analysis. The lifting process was repeated using hinge lifters. As the hinge lifters exhibited strong Raman bands the spectroscopic analysis was more complex and an increase in the number of exposures to the detector facilitated improved clarification. Spectral subtraction was performed to remove peaks due to the hinge lifters using OMNIC software. Developed and lifted fingerprints recorded through evidence bags were were analysed by Raman spectroscopy and it was found that the detection process was not compromised. Whereas the application of powders did not interfere with the detection process, the time taken to locate the contaminant was increased due to the physical presence of more material within the fingerprint

9 Stimulants

Alimpiev S, Grechnikov A, Sunner J*, Borodkov A, Karavanskii V, Simanovsky Y, Nikiforov S// *Univ Portsmouth, Sch Pharm & Biomed Sci, Portsmouth PO1 2DT, England

Anal Chem 2009 **81** (3) 1255

Gas chromatography/surface assisted laser desorption ionization mass spectrometry of amphetamine-like compounds

Gas chromatography/surface-assisted laser desorption ionization mass spectrometry, GC/SALDI-MS was employed to analyse a variety of amphetamine-like compounds. For the SALDI method, compounds are adsorbed on a solid SALDI substrate and directly ionized from the substrate by means of a laser pulse. The interfacing of a SALDI ion source with a gas chromatograph is presented here for the first time. The end of the GC column was situated 20 mm from the silicon substrate in the vacuum of the ion source of a time-of-flight mass spectrometer, and the compounds eluted from the GC capillary were adsorbed onto the nanostructured silicon surface. Very low levels of background noise and no reagent ions were observed in the mass spectra show. GC/SALDI-MS detection limits are several orders of magnitude lower than those previously reported for GC/MS analysis of amphetamine-like compounds. The extent of fragmentation was under experimental control by changing the laser fluence

Maietti S, Castagna F, Molin L, Ferrara SD, Traldi P*// *CNR-ISTM, Corso Stati Uniti 4, IT-135127 Padova, Italy

J Mass Spectrom 2009 **44** (7) 1124

Cocaine adulterants used as marker compounds

No abstract available

Marais AAS, Laurens JB*// *Univ Pretoria, Dept Chem, ZA-0002 Pretoria, Rep Sth Africa

Forensic Sci Int 2009 **183** (1-3) 78

Rapid GC-MS confirmation of amphetamines in urine by extractive acylation

Amphetamine and related derivatives are commonly abused central- and psychostimulants. Detection of certain derivatives, such as methcathinone, by commonly available immunoassay screening techniques is inadequate.

Therefore, multi-analyte analysis for amphetamine type stimulants is required, but traditional gas chromatography-mass spectrometry methods necessitate lengthy analytical procedures with prolonged sample turn-around times. A validated rapid GC-MS assay for urinary identification of amphetamine, methamphetamine, methcathinone, ephedrine, norephedrine, methylenedioxyamphetamine, methylenedioxymethamphetamine, methylenedioxyethylamphetamine and *N*-methyl-1-(3,4 methylenedioxyphenyl)-2-butanamine is described. The technique employs *in situ* derivatization of urine specimens by extractive acylation with pentafluoropropionic anhydride and subsequent rapid chromatography on a microbore capillary column. Analytes were separated in less than 3 min and quantified simultaneously by selected-ion monitoring using stable isotope substituted internal standards. The total instrument cycle-time was 6 min per sample. Limits of detection were between 1.5 ng/ml and 6.25 ng/ml for the various analytes. Intermediate precision and accuracy were in the range of 6.3-13.8% and 90.5-107.3% for the respective analytes at the lower limit of quantitation, and between 5.8-12.6% and 95.4-103.1% for the high control. Long-term storage of methcathinone positive specimens at -20ºC identified inadequate stability of this analyte. The proposed assay is precise and accurate for identification of amphetamine and derivatives in urine. The complementary approach of extractive-derivatization and fast GC-MS analysis is particularly useful in routine clinical settings where reduced sample turn-around times are required. Further investigation of cathinone as a possible metabolite of methcathinone is necessary, based on results from analyzed authentic urine samples

Vogliardi S, Favretto D, Frison G, Ferrara SD*, Seraglia R, Traldi P// *Univ Hosp Padova, Forensic Toxicol & Antidoping Unit, Via Falloppio 50, IT-35121 Padua, Italy

J Mass Spectrom 2009 **44** (1) 18

A fast screening MALDI method for the detection of cocaine and its metabolites in hair

Matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry was used for the rapid detection of cocaine, benzoylecgonine and cocaethylene in hair. Different MALDI sample preparation procedures have been tested and the employment of a multi-layer 'graphite-sample-electrosprayed α-cyano-4-hydroxycinnamic acid (HCCA)' yielded the best results for standard solutions of the target analytes. The same approach was subsequently applied to hair samples that were known to contain cocaine, benzoylecgonine and cocaethylene, as determined by a classical GC-MS method. It was however necessary to extract hair samples by incubating them in methanol/trifluoroacetic acid for a short time (15 min) at 45ºC; 1 µl of the obtained supernatant was deposed on a metal surface treated with graphite, and HCCA was electrosprayed on it. This procedure successfully suppressed matrix peaks and was effective in detecting all the target analytes as their protonated species. The results obtained give further confirmation of the effectiveness of the MALDI for detecting drugs and their metabolites in complex biological matrices. The method can be useful as a fast screening procedure to detect the presence of cocaine and metabolites in hair samples

10 Hallucinogens

Bennett MJ, Steiner RR// Virginia Commonwealth Univ, Dept Forensic Sci, Richmond, Va 23284, USA

J Forensic Sci 2009 **54** (2) 370

Detection of -hydroxybutyric acid in various drink matrices *via* AccuTOF-DART

A new screening method for detecting γ-hydroxybutyric acid (GHB) in drink matrices, using the IonSense, Inc. (Saugus, MA) direct analysis in real time (DART) ion source coupled to a JEOL exact mass time-of-flight mass spectrometer (AccuTOF), was validated and compared with the current screening methodology. The DART ion source allows for analysis of samples under ambient conditions with little to no sample preparation. Fifty drink specimens were spiked at levels of 1, 2, 3, and 4 mg/ml GHB, and analyzed on the AccuTOF-DART. Positive detection of GHB occurred for each of the samples at each concentration level, giving 100% accuracy for the samples tested. Twenty-five of the 50 drink specimens were spiked at 1 mg/ml GHB and tested using a color test known as the GHB Color Test #3. Only two of these 25 specimens tested positive for the presence of GHB, giving only 8% accuracy. Implementation of this new methodology as a screening tool for GHB analysis will quickly eliminate negative specimens allowing the examiner to focus analysis time on those that screened positive

Collins M, Cawley AT, Heagney AC, Kissane L, Robertson J, Salouros H// National Measurement Institute, 1 Suakin Street, Pymble, Sydney, Australia

Rapid Commun Mass Spectrom 2009 **23** (13) 2003

^{13}C, ^{15}N and ^{2}H isotope ratio mass spectrometry of ephedrine and pseudoephedrine: Application to methylamphetamine profiling

Conventional chemical profiling of methylamphetamine has been used for many years to determine the synthetic route employed and where possible to identify the precursor chemicals used. In this study stable isotope ratio analysis was investigated as a means of determining the origin of the methylamphetamine precursors, ephedrine and pseudoephedrine. Ephedrine and pseudoephedrine may be prepared industrially by several routes. Results are presented for the stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and hydrogen ($\delta^{2}H$) measured in methylamphetamine samples synthesized from ephedrine and pseudoephedrine of known provenance. It is clear from the results that measurement of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ stable isotope ratios by elemental analyzer/thermal conversion isotope ratio mass spectrometry (EA/TC-IRMS) in high-purity methylamphetamine samples will allow determination of the synthetic source of the ephedrine or pseudoephedrine precursor as being either of a natural, semi-synthetic, or fully synthetic origin.

Fischedick JT, Glas R, Hazekamp A, Verpoorte R// Leiden Univ, Div Pharmacognosy, Gorlaeus Labs, Rm HB034, Einsteinweg 55, NL-2333 CC Leiden, The Netherlands

Phytochem Anal 2009 **20** (5) 421

A qualitative and quantitative HPTLC densitometry method for the analysis of cannabinoids in *Cannabis sativa* L.

Cannabis and cannabinoid based medicines are currently under serious investigation for legitimate development as medicinal agents, necessitating new low-cost, high-throughput analytical methods for quality control. The goal of this study was to develop and validate, according to ICH guidelines, a simple rapid HPTLC method for the quantification of Δ^9-tetrahydrocannabinol (Δ^9-THC) and qualitative analysis of other main neutral cannabinoids found in cannabis. The method was developed and validated with the use of pure cannabinoid reference standards and two medicinal cannabis cultivars. Accuracy was determined by comparing results obtained from the HTPLC method with those obtained from a validated HPLC method. Δ^9-THC gives linear calibration curves in the range of 50-500 ng at 206 nm with a linear regression of $y = 11.858x + 125.99$ and $r^2 = 0.9968$. Results have shown that the HPTLC method is reproducible and accurate for the quantification of Δ^9-THC in cannabis. The method is also useful for the qualitative screening of the main neutral cannabinoids found in cannabis cultivars

Fu S, Tattam BN, Duke CC, Ramzan I*// *Univ Sydney, Fac Pharm, Sydney, NSW 2006, Australia

Biomed Chromatogr 2009 **23** (1) 81

High-performance liquid chromatography assays for desmethoxyyangonin, methysticin, kavain and their microsomal metabolites

Three novel, simple and reproducible high-performance liquid chromatography quantitative assays with UV detection were developed and validated for three major kavalactones—desmethoxyyangonin, methysticin and kavain—in rat liver microsomes using diazepam as an internal standard; liquid-liquid extraction was used for sample preparation and analysis was performed on a Shimadzu 10A high-performance liquid chromatography system. The analysis was carried out in reversed-phase mode with a Luna C_{18} column (150 x 2.00 mm, 3 µm) at 40ºC. The limit of quantitation was 0.1 µg/ml using 0.25 ml of microsomal solution. The assays were linear over the range 0.1-10 µg/ml for desmethoxyyangonin, methysticin and kavain. Quality control samples exhibited good accuracy and precision with relative standard deviations lower than 15% and recoveries between 85 and 105%. The assays exhibited satisfactory performance with high sensitivity for quantifying desmethoxyyangonin, methysticin and kavain in rat liver microsomes and were successfully used to determine the three kavalactones and their microsomal metabolites

Inoue H, Hashimoto H, Watanabe S, Iwata YT, Kanamori T, Miyaguchi H, Tsujikawa K, Kuwayama K, Tachi N, Uetake N// Nat Res Inst Police Sci, First Chem Sect, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277 0882, Japan

J Mass Spectrom 2009 **44** (9) 1300

Thermal desorption counter-flow introduction atmospheric pressure chemical ionization for direct mass spectrometry of ecstasy tablets

A novel approach to the analysis of ecstasy tablets by direct mass spectrometry coupled with thermal desorption (TD) and counter-flow introduction atmospheric pressure chemical ionization (CFI-APCI) is described. Analytes were thermally desorbed with a metal block heater and introduced to a CFI-APCI source with ambient air by a diaphragm pump. Water in the air was sufficient to act as the reactive reagent responsible for the generation of ions in the positive corona discharge. TD-CFI-APCI required neither a nebulizing gas nor solvent flow and the accompanying laborious optimizations. Ions generated were sent in the direction opposite to the air flow by an electric field and introduced into an ion trap mass spectrometer. The major ions corresponding to the protonated molecules ($[M + H]^+$) were observed with several fragment ions in full scan mass spectrometry (MS) mode. Collision-induced dissociation of protonated molecules gave characteristic product-ion mass spectra and provided identification of the analytes within 5 s. The method required neither

sample pretreatment nor a chromatographic separation step. The effectiveness of the combination of TD and CFI-APCI was demonstrated by application to the direct mass spectrometric analysis of ecstasy tablets and legal pharmaceutical products

Jagerdeo E, Schaff JE, Montgomery MA, LeBeau MA// FBI Lab, 2501 Investigation Parkway, Quantico, Va 22135, USA
Rapid Commun Mass Spectrom 2009 **23** (17) 2697
A semi-automated solid-phase extraction liquid chromatography/tandem mass spectrometry method for the analysis of tetrahydrocannabinol and metabolites in whole blood
Marijuana is one of the most commonly abused illicit substances in the USA, making cannabinoids important to detect in clinical and forensic toxicology laboratories. Historically, cannabinoids in biological fluids have been derivatized and analyzed by gas chromatography/mass spectrometry (GC/MS). There has been a gradual shift in many laboratories towards liquid chromatography/mass spectrometry (LC/MS) for this analysis due to its improved sensitivity and reduced sample preparation compared with GC/MS procedures. This paper reports a validated method for the analysis of Δ^9-tetrahydrocannabinol (THC) and its two main metabolites, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) and 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), in whole blood samples. The method has also been validated for cannabinol (CBD) and cannabidiol (CDN), two cannabinoids that were shown not to interfere with the method. This method has been successfully applied to samples both from living people and from deceased individuals obtained during autopsy. This method utilizes online solid-phase extraction (SPE) with LC/MS. Pretreatment of samples involves protein precipitation, sample concentration, ultracentrifugation, and reconstitution. The online SPE procedure was developed using Hysphere C8-EC sorbent. A chromatographic gradient with an Xterra MS C_{18} column was used for the separation. Four multiple-reaction monitoring (MRM) transitions were monitored for each analyte and internal standard. Linearity generally fell between 2 and 200 ng/ml. The limits of detection (LODs) ranged from 0.5 to 3 ng/ml and the limits of quantitation (LOQs) ranged from 2 to 8 ng/ml. The bias and imprecision were determined using a simple analysis of variance (ANOVA: single factor). The results demonstrate bias as <7%, and imprecision as <9%, for all components at each quantity control level

Jermain JD, Evans HK// US Dept Justice, Bureau Alcohol Tobacco Firearms & Explosives, Forensic Sci Lab, 355 Nth Wiget Lane, Walnut Creek, Ca 94598, USA
J Forensic Sci 2009 **54** (3) 612
Analyzing *Salvia divinorum* and its active ingredient salvinorin A utilizing thin layer chromatography and gas chromatography/mass spectrometry
In recent years, *Salvia divinorum* has become a major focus by state legislatures throughout the United States looking to prohibit the sale of the psychoactive plant. After researching testing procedures presented in the literature and those employed by crime laboratories throughout the country, it was decided that thin layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS) were the methods to use to analyze plant material for salvinorin A. With TLC, salvinorin A was detected from extracted plant material and was easily distinguishable from 13 other *Salvia* species as well as *Cannabis sativa* L. (marijuana). When using GC/MS, salvinorin A was best extracted from plant material with chloroform at ambient temperature when using a nonpolar solvent and acetone at ambient temperature when using a polar solvent. By utilizing these techniques, criminalists are now able to confirm the presence of salvinorin A in a submitted plant material suspected to be *Salvia divinorum*

Mendoza MA, Mills DK, Lata H, Chandra S, ElSohly MA, Almirall JR*// *Florida Int Univ, Dept Chem & Biochem, University Park, Miami, Fl 33199, USA
Anal Bioanal Chem 2009 **393** (2) 719
Genetic individualization of *Cannabis sativa* by a short tandem repeat multiplex system
In the USA, *Cannabis sativa* is the most frequently used of all illicit drugs. Short tandem repeats (STRs) were chosen as molecular markers owing to their distinct advantages over other genetic methods. STRs are codominant, may be standardized such that reproducibility between laboratories may be readily achieved, have a high discrimination power, and may be multiplexed. Six STR markers previously described for *C. sativa* were multiplexed into one reaction. The multiplex reaction was able to individualize 98 cannabis samples (14 hemp and 84 marijuana, authenticated as originating from 33 of the 50 states of the USA) and detect 29 alleles averaging 4.8 alleles per loci.

Miyaguchi H, Takahashi H, Ohashi T, Mawatari K, Iwata YT, Inoue H, Kitamori T// Natl Res Inst Police Sci, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277 0882, Japan
Forensic Sci Int 2009 **184** (1-3) 1
Rapid analysis of methamphetamine in hair by micropulverized extraction and microchip-based competitive ELISA
A microchip-based ELISA system (microELISA) in combination with a micropulverized extraction method has been developed as an automated full-range quantitation technique for identifying *d*-methamphetamine in human hair. The competitive ELISA assay employed an antibody and a peroxidase-linked methamphetamine, both of which are commercially available. To verify the reliability and applicability of this new method, validation was carried out using doped hair samples, and segmental analyses of real-case specimens were carried out by both microELISA and LC/MS/MS. The small size of the system and the lack of an evaporation process facilitated sample preparation and quantitation was easily and rapidly accomplished in less than 30 min

Mueller M, Peters FT, Huestis MA, Ricaurte GA, Maurer HH*// *Univ Saarland, Inst Expt & Clin Pharmacol & Toxicol, Dept Expt & Clin Toxicol, DE-66421 Homburg, Saar, Germany
Forensic Sci Int 2009 **184** (1-3) 64
Simultaneous liquid chromatographic-electrospray ionization mass spectrometric quantification of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and its metabolites 3,4-dihydroxymethamphetamine, 4 hydroxy-3 methoxymethamphetamine 3,4-methylenedioxyamphetamine in squirrel monkey and human plasma after acidic conjugate cleavage
3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) is a psychoactive drug with both abuse and neurotoxic potential. An LC-MS assay with electrospray ionization for quantifying MDMA and its main metabolites in squirrel monkey plasma was modified to include acidic hydrolysis to obtain total 3,4-dihydroxymethamphetamine and 4-hydroxy-3-methoxy-methamphetamine. Re-validation for squirrel monkey plasma and full validation for human plasma illustrated selectivity for all analytes. Recoveries were > or = 71.0%. Changed specimen preparation or matrix did not affect accuracy or precision. No instability was noted following repeated freezing or in processed samples. Quantification of plasma MDMA and metabolites, derived pharmacokinetic and toxicokinetic data and neurotoxicity research will be enhanced by this validated method

Nieddu M, Boatto G*, Pirisi MA, Baralla E// *Univ Sassari, Dipt Farmaco Chim Tossicol, Via Muroni 23, IT-07100 Sassari, Italy
Rapid Commun Mass Spectrom 2009 **23** (19) 3051
Multi-residue analysis of eight thioamphetamine designer drugs in human urine by liquid chromatography/tandem mass spectrometry
An analytical procedure for the simultaneous determination in human urine of several thioamphetamine designer drugs (2C-T and ALEPH series) is reported. The quantitative analysis was performed by liquid chromatography/tandem mass spectrometry and has been fully validated. The mass spectrometer was operated in positive-ion, selected reaction monitoring (SRM) mode. In order to minimize interferences with matrix components and to preconcentrate target analytes, solid-phase extraction was introduced in the method as a clean-up step. The entire method was validated for selectivity, linearity, precision and accuracy. The method turned out to be specific, sensitive, and reliable for the analysis of amphetamine derivatives in urine samples. The calibration curves were linear over the concentration range of 1 to 100 ng/ml for all drugs with correlation coefficients that exceeded 0.996. The lower limits of detection (LODs) and quantification (LOQs) ranged from 1.2 to 4.9 ng/ml and from 3.2 to 9.6 ng/ml, respectively

Pires APS, Oliveira CDRD, Moura S, Dorr FA, Silva WAE, Yonamine M*// *Univ Sao Paulo, Fac Pharmaceut Sci, Dept Clin & Toxicol Anal, Av Prof Lineu Prestes 580 B13B, BR-05508-900 Sao Paulo, Brazil
Phytochem Anal 2009 **20** (2) 149
Gas chromatographic analysis of dimethyltryptamine and -carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage
Ayahuasca is obtained by infusing the pounded stems of *Banisteriopsis caapi* in combination with the leaves of *Psychotria viridis*. *P. viridis* is rich in the psychedelic indole *N*,*N*-dimethyltryptamine, whereas *B. caapi* contains substantial amounts of β-carboline alkaloids, mainly harmine, harmaline and tetrahydroharmine, which are monoamine-oxidase inhibitors. Because of differences in composition in ayahuasca preparations, a method to measure their main active constituents is needed. To develop a gas chromatographic method for the simultaneous determination of dimethyltryptamine and the main β-carbolines found in ayahuasca preparations. The alkaloids were extracted by means of solid phase extraction (C_{18}) and detected by gas chromatography with nitrogen/phosphorous detector. The lower limit of quantification (LLOQ) was 0.02 mg/ml for all analytes. The calibration curves were linear over a concentration range of 0.02-4.0 mg/ml (r^2 > 0.99). The method was also precise (RSD < 10%). A simple gas chromatographic method to determine the main alkaloids found in ayahuasca was developed and validated. The method can be useful to estimate administered doses in animals and humans for further pharmacological and toxicological investigations of ayahuasca

West JB, Hurley JM, Ehleringer JR// Univ Utah, Dept Biol, 257 Sth 1400 East, Salt Lake City, Ut 84112, USA

J Forensic Sci 2009 **54** (1) 84

Stable isotope ratios of marijuana. I. Carbon and nitrogen stable isotopes describe growth conditions

There remains significant uncertainty in illicit marijuana cultivation. We analyzed the $\delta^{13}C$ and $\delta^{15}N$ of 508 domestic samples from known U.S.A. counties, 31 seized from a single location, 5 samples grown in Mexico and Colombia, and 10 northwest border seizures. For a subset, inflorescences and leaves were analyzed separately. These data revealed a strong correspondence, with inflorescences having slightly higher $\delta^{13}C$ and $\delta^{15}N$ values than leaves. A framework for interpreting these results is introduced and evaluated. Samples identified as outdoor-grown by $\delta^{13}C$ were generally recorded as such by the Drug Enforcement Administration (DEA). DEA-classified indoor-grown samples had the most negative $\delta^{13}C$ values, consistent with indoor cultivation, although many were also in the outdoor-grown domain. $\delta^{15}N$ indicated a wide range of fertilizers across the dataset. Samples seized at the single location suggested multiple sources. Northwest border $\delta^{13}C$ values suggested indoor growth, whereas for the Mexican and Colombian samples they indicated outdoor growth

11 Narcotics

Casale JF, Toske SG, Hays PA// US Dept Justice, Drug Enforcement Admin, Special Testing & Res Lab, Dulles, Va 20166, USA

J Forensic Sci 2009 **54** (2) 359

Chlorinated opium alkaloid derivatives produced by the use of aqueous sodium hypochlorite during the clandestine manufacture of heroin

A clandestine chemist was observed producing heroin from crude morphine utilizing a solution of sodium hypochlorite during the process. Numerous chlorinated opium alkaloid derivatives were created when the morphine acetylation reaction was quenched and neutralized with a solution of sodium hypochlorite and ammonium hydroxide. Four of these compounds, 1-chloroheroin, 1-chloroacetylcodeine, 1-chloro-O^6-monoacetylmorphine, and 2'-chloropapaverine, were characterized *via* preparative isolation, gas chromatography/mass spectrometry, nuclear magnetic resonance spectroscopy, and independent synthesis. These chlorinated derivatives were formed *via* electrophilic aromatic substitution with free chlorine during the illicit process. Although no illicit heroin exhibits containing these compounds have been observed in seizures to date, mass spectral data are provided for several of these compounds for their identification should they be seen within future seizures of illicit heroin

Harding G// GE Security Germany GmbH, Heselstuecken 3, DE-22453 Hamburg, Germany

Appl Radiat Isot 2009 **67** (2) 287

X-ray diffraction imaging—A multi-generational perspective

Some applications of X-ray diffraction imaging (XDI) in security screening, including detection of narcotics and a wide range of explosives: organic (plastic) explosives, liquids, home-made explosives (HMEs) and special nuclear materials (SNMs) is described. A Bayesian formulation of the "rare event scenario" is presented, allowing the probability to be quantified that an unlikely threat is indeed present when an uncertain detection system raises an alarm. Granted the utility of X-ray diffraction (XRD) as a significant screening modality for false-alarm resolution, its technological feasibility is presented. When compared with computed tomography, XDI permits a significant reduction to be achieved in measurement time per object volume element (voxel) compared with that of a classical X-ray diffractometer. This reduction may be achieved by designing the XDI system to record energy-dispersive XRD profiles from many volume elements (object voxels) in parallel. A general scheme for designing "massively-parallel" (MP) XDI systems is presented. XDI configurations of the first generation (1 voxel/s), second generation (100 voxels/s) and third generation (10^4 voxels/s) are presented and discussed. Three alternative third generation XDI geometries, namely: direct fan-beam; parallel (waterfall) beam; and inverse fan-beam were compared in respect of technological realization. Directions for future development of XDI in screening applications are provided

Huang DK, Liu C*, Huang MK, Chien CS// *Nat Bureau Controlled Drugs, Dept Hlth, 6 Linsen Sth Rd, Taipei 10050, Taiwan

Rapid Commun Mass Spectrom 2009 **23** (7) 957

Simultaneous determination of morphine, codeine, 6-acetylmorphine, cocaine and benzoylecgonine in hair by liquid chromatography/electrospray ionization tandem mass spectrometry

A fast and sensitive liquid chromatography/triple quadrupole tandem mass spectrometry (LC/MS/MS) method was developed for the simultaneous determination of morphine, codeine, 6-acetylmorphine (6-AM), cocaine and benzoylecgonine (BE) in hair. Pulverized hair samples were extracted with methanol, and a 50 µl supernatant aliquot was injected into the LC/MS/MS system. Chromatography was performed with an XBridge phenyl column (3.5 µm particle size, 4.6 x 150 mm), and the mobile phase was composed of methanol and 10 mM ammonium acetate adjusted to pH 4.00 with 99% formic acid (95:5, v/v). A separation run with isocratic elution was completed in 10 min at a flow rate of 500 µl/min. Positive electrospray ionization and multiple reaction monitoring (MRM) with one precursor ion/product ion transition were used for the identification of each analyte. Deuterated analogues as internal standards were used for quantification and qualification. Linearity was established in the concentration range of 100-3000 pg/mg. The limits of detection were 10 pg/mg for morphine, codeine and 6-AM; and 1 pg/mg for cocaine and BE. The precision and accuracy were determined by spiking hair samples at six concentration levels. For all analytes, the relative standard deviations of intra- and inter-day precision were 0.1-6.3% and 1.5-10.6%, respectively. The accuracy ranged from 92.7 to 109.7%. The validated LC/MS/MS method was successfully applied to the analysis of 79 authentic hair samples

Lee S, Cordero R, Paterson S*// *Imperial Coll London, Toxicol Unit, St Dunstans Rd, London W6 8RP, England

Forensic Sci Int 2009 **183** (1-3) 74

Distribution of 6-monoacetylmorphine and morphine in head and pubic hair from heroin-related deaths

This study was conducted to collate 6-monoacetylmorphine (6-AM) and morphine concentrations in head and pubic hair from heroin users and to propose reference ranges and relate these to the amount of heroin used. The ratio of morphine:6-AM was also examined. A total of 82 head hair samples divided into 173 segments of various lengths and 15 pubic hair samples were collected at postmortem from heroin users. Statistical analysis demonstated that in head hair, the lower, middle and upper concentration ranges of 6-AM were 0.1-0.9, 0.9-12.5 and 12.5-154.1 ng/mg and those of morphine were 0.1-0.8, 0.8-6.0 and 6.0-36.3 ng/mg. In pubic hair, the lower, middle and upper concentration ranges of 6-AM were 0.2-0.5, 0.5-2.3 and 2.3-18.2 ng/mg and those of morphine were 0.2-0.4, 0.4-2.4 and 2.4-13.3 ng/mg. The morphine:6-AM ratio showed a large variation. The ratio tended to decrease from proximal to distal segments. Statistical analysis provides low, middle and high concentration ranges which may be employed for estimating the amount of heroin consumed into corresponding low or occasional, regular or habitual and heavy or excessive drug use. The ratio of morphine:6-AM showed great variation and did not support a ratio less than 0.77 is necessary to prove ingestion of heroin

Philipp AA, Wissenbach DK, Zoerntlein SW, Klein ON, Kanogsunthornrat J, Maurer HH*// *Saarland Univ, Dept Experimental & Clin Toxicol, DE-66421 Homburg/Saar, Germany

J Mass Spectrom 2009 **44** (8) 1249

Studies on the metabolism of mitragynine, the main alkaloid of the herbal drug Kratom, in rat and human urine using liquid chromatography-linear ion trap mass spectrometry

Mitragynine (MG) is an indole alkaloid of the Thai medicinal plant *Mitragyna speciosa* (Kratom in Thai) and reported to have opioid agonistic properties. Because of its stimulant and euphoric effects, Kratom is used as a herbal drug of abuse. The aim of the presented study is to identify the phase I and II metabolites of MG in rat and human urine after solid-phase extraction (SPE) using liquid chromatography-linear ion trap mass spectrometry providing detailed structure information in the MS^n mode particularly with high resolution. The seven identified phase I metabolites indicated that MG was metabolized by hydrolysis of the methylester in position 16, *O*-demethylation of the 9-methoxy group and of the 17-methoxy group, followed, *via* the intermediate aldehydes, by oxidation to carboxylic acids or reduction to alcohols and combinations of some steps. In rats, four metabolites were additionally conjugated to glucuronides and one to sulfate, but in humans, three metabolites to glucuronides and three to sulfates

Subhan F, Khan N, Sewell RDE*// *Welsh Sch Pharm, Div Pharmacol, Redwood Bldg, King Edward 7 Ave, Cardiff CF10 3NB, Wales

Phytother Res 2009 **23** (4) 564

Adulterant profile of illicit street heroin and reduction of its precipitated physical dependence withdrawal syndrome by extracts of St John's wort (*Hypericum perforatum*)

The study evaluated the adulterants in a specimen of illicit street heroin supplied under strict control by the Pakistan Anti-Narcotic Force. It also examined the effects of *Hypericum perforatum* L. extracts on the naloxone-induced heroin withdrawal syndrome. The GC-MS analysis of the specimen showed that in addition to heroin (37.8%), the sample also contained caffeine (8.4%), phenobarbitone (12.7%), 6-acetyl codeine (5.3%), 6-acetyl morphine (10.9%) and noscapine (15.8%). Administration of the heroin to rats for 8 days induced physical withdrawal signs of abdominal constriction, diarrhoea and vocalization on touch after naloxone treatment. Aqueous *Hypericum perforatum* extracts (20 mg/kg twice daily chronically or as a single acute dose 90 min before naloxone) given orally to the heroin dependent rats attenuated abdominal constrictions both acutely and chronically while the hydroethanol and ethanol extracts were only effective in acutely treated animals. Diarrhoea was ameliorated by the hydroethanol and ethanol extracts following acute or

chronic heroin treatment while the aqueous extract failed to show any effect. Vocalization on touch during withdrawal was reduced by all the extracts either chronically or acutely with the exception of chronic treatment with hydroethanol extracts. The findings suggest that *Hypericum perforatum* is capable of reducing the physical signs of opiate withdrawal

Zhang ZX, Yan B, Liu KL, Liao YP, Liu HW*// *Peking Univ, Coll Chem & Mol Engn, MoE Key Lab Bioorgan Chem & Mol Engn, CN-100871 Beijing, Peoples Rep China

Electrophoresis 2009 **30** (2) 379

CE-MS analysis of heroin and its basic impurities using a charged polymer-protected gold nanoparticle-coated capillary

The first application of charged polymer-protected gold nanoparticles (Au NPs) as semi-permanent capillary coating in CE-MS was presented. Poly(diallyldimethylammonium chloride) (PDDA) was the only reducing and stabilizing agent for Au NPs preparation. Stable and repeatable coating with good tolerance to 0.1 M HCl, methanol, and ACN was obtained *via* a simple rinsing procedure. Au NPs enhanced the coating stability toward flushing by methanol, improved the run-to-run and capillary-to-capillary repeatabilities, and improved the separation efficiency of heroin and its basic impurities for tracing geographical origins of illicit samples. Baseline resolution of eight heroin-related alkaloids was achieved on the PDDA-protected Au NPs-coated capillary under the optimum conditions: 120 mM ammonium acetate (pH 5.2) with addition of 13% methanol, separation temperature 20ºC, applied voltage -20 kV, and capillary effective length 60.0 cm. CE-MS analysis with run-to-run RSDs (n=5) of migration time in the range of 0.43-0.62% and RSDs (n=5) of peak area in the range of 1.49-4.68% was obtained. The established CE-MS method would offer sensitive detection and confident identification of heroin and related compounds and provide an alternative to LC-MS and GC-MS for illicit drug control

12 Forensics

Auwarter V, Dresen S, Weinmann W, Muller M, Putz M, Ferreiros N// Univ Med Ctr Freiburg, Inst Forensic Med, Forensic Toxicol, Albertstr 9, DE-79104 Freiburg, Germany

J Mass Spectrom 2009 **44** (5) 832

'Spice' and other herbal blends: Harmless incense or cannabinoid designer drugs?

No abstract available

Bin Abdullah AFL, Miskelly GM// Univ Auckland, Forensic Sci Programme, Private Bag 92 019, Auckland 1, New Zealand

J Forensic Sci 2009 **54** (2) 365

Formation of trifluoroacetylated ephedrine during the analysis of a pseudoephedrine-formaldehyde adduct by TFAA derivatization followed by GC-MS

(+)-Pseudoephedrine reacts with formaldehyde to form (4*S*,5*S*)-3,4-dimethyl-5-phenyloxazolidine. Gas chromatography-mass spectrometry (GC-MS) analysis after the reaction of this oxazolidine with excess trifluoroacetic acid anhydride (TFAA) shows predominantly *N*,*O*-bis(trifluoroacetyl)pseudoephedrine with some of the monotrifluoroacetylated derivative. In addition, variable amounts of *N*,*O*-bis(trifluoroacetyl)ephedrine were detected by GC-MS. *N*,*O*-bis(trifluoroacetyl)ephedrine was not detected upon trifluoroacetylation of the source (+)-pseudoephedrine, and nuclear magnetic resonance analysis of the (4*S*,5*S*)-3,4-dimethyl-5-phenyloxazolidine showed no evidence of the (4*R*,5*S*) isomer. This suggests that the *N*,*O*-bis(trifluoroacetyl)ephedrine is formed by epimerization during the TFAA derivatization and GC-MS analysis of the pseudoephedrine-formaldehyde adduct

Li H, Ricordel I, Tong L, Schopfer LM, Baud F, Megarbane B, Maury E, Masson P, Lockridge O*// *Eppley Inst, 986805 Nebraska Med Ctr, Omaha, Ne 68198, USA

J Appl Toxicol 2009 **29** (2) 149

Carbofuran poisoning detected by mass spectrometry of butyrylcholinesterase adduct in human serum

Carbofuran is a pesticide whose acute toxicity is due to inhibition of acetylcholinesterase. Butyrylcholinesterase (BChE) in plasma is inhibited by carbofuran and serves as a biomarker of poisoning by carbofuran. The goal was to develop a method to positively identify poisoning by carbofuran. Sera from an attempted murder and an attempted suicide were analyzed for the presence of carbofuran adducts on BChE. The BChE from 1 ml of serum was rapidly purified on a 0.2 ml procainamide-Sepharose column. Speed was essential because the carbofuran-BChE adduct decarbamylates with a half-life of about 2 h. The partially purified BChE was boiled to denature the protein, thus stopping decarbamylation and making the protein vulnerable to digestion with trypsin. The labeled peptide was partially purified by HPLC before analysis by LC/MS/MS in the multiple reaction monitoring mode on the QTRAP 2000 mass spectrometer. Carbofuran was found to be covalently bound to Ser 198 of human BChE in serum samples from two poisoning cases. Multiple reaction monitoring triggered MS/MS spectra positively identified the carbofuran-BChE adduct. In conclusion a mass spectrometry method to identify carbofuran poisoning in humans has been developed. The method uses 1 ml of serum and detects low-level exposure associated with as little as 20% inhibition of plasma butyrylcholinesterase

Park MJ, In SW, Lee SK, Choi WK, Park YS, Chung HS// Natl Inst Sci Invest, Dept Forensic Sci, 331-1 Shinwol 7 dong, Yangcheon gu, Seoul, South Korea

Forensic Sci Int 2009 **184** (1-3) 28

Postmortem blood concentrations of organophosphorus pesticides

Acute fatalities due to ingestion of organophosphorus pesticides (OPs), such as chlorpyrifos, diazinon, malathion and parathion, are described. The analysis of OPs in postmortem blood was achieved with solid-phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS). After extraction with an Oasis HLB cartridge, the eluent was evaporated to dryness under a nitrogen stream at 35ºC, reconstituted with ethanol, and then analyzed by GC/MS. Terbufos was used as an internal standard. Limit of detection, limit of quantification, linearity of the calibration, precision and recovery were carried out for verification. Validation data were adequate for analyzing OPs in blood. Chlorpyrifos, diazinon, malathion and parathion were identified in 31 postmortem blood samples. Parathion was the most frequently detected compound among the four pesticides. The mean concentrations of chlorpyrifos, diazinon, malathion and parathion were 0.72, 1.03, 0.82 and 2.90 mg/l, respectively

Saar E, Gerostamoulos D, Drummer OH, Beyer J*// *Monash Univ, Victorian Inst Forensic Med, Dept Forensic Med, 57-83 Kavanagh St, Southbank, Australia

Anal Bioanal Chem 2009 **393** (2) 727

Comparison of extraction efficiencies and LC-MS-MS matrix effects using LLE and SPE methods for 19 antipsychotics in human blood

Sudden death investigations frequently involve antipsychotic drugs. Identification of these drugs is required to establish their use and possible contribution to the death. LC-MS(MS) methods are often employed. However accurate and precise quantification is confirmed using validated methods. Extraction efficiency and matrix effects using common liquid-liquid and solid-phase extraction procedures in both ante-mortem and post-mortem specimen using LC-MS-MS have been compared. Extraction efficiencies and matrix effects were determined in five different blank blood specimens of each blood type. Samples were extracted using a number of different liquid-liquid extraction methods and compared with a standard mixed-mode solid-phase extraction method. A post-extraction addition approach was employed to ivestigate matrix effects. Blank blood specimens were extracted as described above and the extracts were reconstituted in mobile phase containing a known amount of analytes. The extraction efficiency was quite different between ante-mortem and post-mortem blood. Quantitative methods used for analysis of antipsychotic drugs in post-mortem blood should establish that there are no differences in extraction efficiency and matrix effects, particularly if employing ante-mortem blood as calibrator

Steiner RR, Larson RL// Virginia Dept Forensic Sci, Cent Lab Drug Anal Sect, 700 Nth 5th St, Richmond, Va, USA

J Forensic Sci 2009 **54** (3) 617

Validation of the direct analysis in real time source for use in forensic drug screening

The Direct Analysis in Real Time (DART) ion source is a relatively new mass spectrometry technique that is seeing widespread use in chemical analyses world-wide. DART studies include such diverse topics as analysis of flavors and fragrances, melamine in contaminated dog food, differentiation of writing inks, characterization of solid counterfeit drugs, and as a detector for planar chromatography. Validation of this new technique for the rapid screening of forensic evidence for drugs of abuse, utilizing the DART source coupled to an accurate mass time-of-flight mass spectrometer, was conducted. The study consisted of the determination of the lower limit of detection for the method, determination of selectivity and a comparison of this technique to established analytical protocols. Examples of DART spectra are included. The results of this study have allowed the Virginia Department of Forensic Science to incorporate this new technique into their analysis scheme for the screening of solid dosage forms of drugs of abuse

Watterson JH, Botman JE// Laurentian Univ, Dept Forensic Sci, Forensic Toxicol Res Lab, 935 Ramsey Lake Rd, Sudbury, Ontario, Canada P3E 2C6

J Forensic Sci 2009 **54** (3) 708

Detection of acute diazepam exposure in bone and marrow: Influence of tissue type and the dose-death interval on sensitivity of detection by ELISA with liquid chromatography tandem mass spectrometry confirmation

Enzyme-linked immunosorbent assay (ELISA) and liquid chromatography tandem mass spectrometry (LC/MS/MS) were used to detect diazepam exposure in skeletal tissues of rats (n = 15) given diazepam acutely (20 mg/kg, i.p.), and killed at various times postdose. Marrow, epiphyseal, and diaphyseal bone were isolated from extracted femora. Bone was cleaned, ground, and incubated in methanol. Marrow underwent ultrasonic homogenization. Extracts and homogenates were diluted in phosphate buffer, and then underwent solid-phase extraction and ELISA. Relative sensitivity of detection was examined in terms of relative decrease in absorbance (ELISA) and binary classification sensitivity (ELISA and LC/MS/MS). Overall, the data showed differences in relative sensitivity of detection of diazepam exposure in different tissue types (marrow > epiphyseal bone > diaphyseal bone), which is suggestive of heterogenous distribution in these tissues, and a decreasing sensitivity with increasing dose-death interval. Thus, the tissue type sampled and dose-death interval may contribute to the probability of detection of diazepam exposure in skeletal tissues

13 Alcohol

Alvarez I, Bermejo AM*, Taherno MJ, Fernandez P, Cabarcos P, Lopez P// *Fac Med, Inst Legal Med, Forensic Toxicol Service, C/ San Francisco s/n, ES-15782 Santiago de Compostela, Spain

Anal Bioanal Chem 2009 **393** (4) 1345

Microwave-assisted extraction: A simpler and faster method for the determination of ethyl glucuronide in hair by gas chromatography-mass spectrometry

Ethyl glucuronide (EtG) is a marker of recent alcohol consumption that detects alcohol use reliably over a definite time period. The determination of EtG in hair is described. The technique involves microwave-assisted extraction (MAE), to extract the analyte from hair samples, and gas chromatography-mass spectrometry (GC-MS), to identify and quantify the EtG in selected ion monitoring (SIM) mode. Fifteen hair samples from occasional alcohol users were analysed and positive results obtained in all cases. It was fully validated, including a linear range (0.3-10 ng/mg) and the main precision parameters. Microwave-assisted extraction is a substantially simpler, faster, and a more sensitive procedure than other conventional sample preparations

De Benedetto GE, Fanigliulo M// Univ Salento, Dipt Beni Arti & Storia, Lab Anal Chim Ambiente & Beni Culturali, IT-73100 Lecce, Italy

Electrophoresis 2009 **30** (10) 1798

A new CE-ESI-MS method for the detection of stable hemoglobin acetaldehyde adducts, potential biomarkers of alcohol abuse

A new CE-ESI-MS method was developed to provide a simple way to study changes to hemoglobin (HbA) induced by acetaldehyde (Ach) *in vitro*. Instrumental parameters were univariately optimized in order to maximize the sensitivity of the CE-ESI-MS method. The electrophoretic separations were carried out in poly-E323-coated capillaries using 60 mM formic acid raised to pH 3.0 with ammonia and containing 5% 2-propanol while the sheath liquid, 2-propanol/water (30:70) with 0.1% formic acid, was delivered at 1.0 µl/min through a coaxial sheath flow electrospray interface. The HbA was incubated with Ach for intervals up to 24 h at concentration varying in the window 0.2-20 mM. Four stable Ach-hemoglobin adducts in the hemoglobin tryptic digest were observed at the submillimolar Ach concentration and characterized by MS/MS experiments: although the α and β N-amino terminal modifications were expected, the two internal ones arising, respectively, from the condensation of Ach molecules on the histidine residue in position 4 in α4 (i.e. the fourth peptide after tryptic digestion of α chain starting from amino terminal) and on the asparagine residue in position 2 in β3, were identified for the first time. During the *in vitro* experiments higher concentrations of Ach were also used; however, it was not possible to identify any other stable modification of hemoglobin. Interestingly, those stable modifications are the only ones *in vivo* identified in the hemoglobin of moderate alcohol drinkers

Gnann H, Weinmann W*, Engelmann C, Wurst FM, Skopp G, Winkler M, Thierauf A, Auwarter V, Dresen S, Bouzas NF// *Univ Med Ctr, Inst Legal Med, Albertstr 9, DE-79104 Freiburg, Germany

J Mass Spectrom 2009 **44** (9) 1293

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

A new validated method for the quantitation of the abnormal phospholipid phosphatidylethanol (PEth)—a biomarker for ethanol uptake—has been developed by LC-ESI-MS/MS following miniaturised organic solvent extraction and reversed phase chromatography with phosphatidylbutanol (PBut) as internal standard. PEth homologues with two fatty acid substituents—PEth 18:1/18:1, PEth 16:0/16:0—were determined in post-mortem blood collected from heavy drinkers at autopsy and also in whole blood samples from a volunteer after a single 60 g-dose of ethanol. Furthermore, PEth 18:1/16:0 or its isobaric isomer PEth-16:0/18:1 was detected. In comparison to previous high-performance liquid chromatography (HPLC) methods with evaporative light scattering detection (ELSD), the LC-MS/MS-method is more sensitive—with a limit of detection below 20 ng/ml—and more selective for single PEth homologues, while ELSD has been used for detection of the sum of PEth homologues with approximately 10 times less sensitivity. LC-MS/MS enables monitoring of PEth homologues as biomarkers for harmful and prolonged alcohol consumption as with HPLC/ELSD earlier, where PEth is measurable in blood only after more than 50 g ethanol daily intake for more than 2 weeks. Because of its higher sensitivity, there is a potential to detect single heavy drinking by LC-MS/MS, when PEth is formed in very low concentrations. This opens a new field of application of PEth to uncover single or multiple heavy drinking at a lower frequency and with a larger window of detection in blood than before by HPLC/ELSD or by use of other direct markers, e.g. ethyl glucuronide or ethyl sulfate

Hoiseth G, Morini L, Polettini A, Christophersen A, Morland J// Norwegian Inst Publ Hlth, Div Forensic Toxicol & Drug Abuse, PB 4404 Nydalen, NO-0403 Oslo, Norway

Alcohol Clin Exp Res 2009 **33** (5) 812

Ethyl glucuronide in hair compared with traditional alcohol biomarkers—A pilot study of heavy drinkers referred to an alcohol detoxification unit

Traditional biomarkers for heavy alcohol use include serum carbohydrate-deficient transferrin (CDT), the enzymes aspartate aminotransferase (AST), and alanine aminotransferase (ALT) as well as γ-glutamyl transferase (GGT). Measurement of the nonoxidative ethanol metabolite, ethyl glucuronide (EtG) in hair, has been proposed as a new marker with superior qualities. The aim of this study was to investigate the sensitivity of EtG in hair to detect heavy alcohol use compared with CDT, AST, ALT, and GGT. We also wanted to study the quantitative relation between alcohol intake and the different biomarkers. Sixteen patients with a history of heavy alcohol use over the previous 3 months were recruited directly after admission to a withdrawal clinic. They were thoroughly interviewed about their drinking pattern as well as relevant diseases and use of medicines or drugs. Serum was sampled and analyzed for %CDT, AST, ALT, and GGT. Hair samples were collected and analyzed for EtG. The mean estimated daily intake (EDI) over the previous 3 months was 206 +/- 136 g pure alcohol. All patients fulfilled the criteria for heavy alcohol use. The sensitivity to detect heavy alcohol use was 64% for %CDT, 67% for AST, 67% for ALT, 93% for GGT, and 94% for EtG. There was no correlation between the quantitative values of EDI and %CDT, AST, ALT, and GGT. There was a positive, statistically significant correlation between EDI and the level of EtG in hair. In this study, EtG in hair and GGT showed the best sensitivity to detect heavy alcohol use and there was a positive correlation between EDI and the concentrations of EtG in hair. Before giving recommendations for clinical practice, further studies should be carried out on larger materials and populations with a wider range of alcohol intake

Junghanns K, Graf I, Pfluger J, Wetterling G, Ziems C, Ehrenthal D, Zollner M, Dibbelt L, Backhaus J, Weinmann W, Wurst FM// Univ Lubeck, Dept Psychiat & Psychotherapy, Ratzeburger Allee 160, DE-23538 Lubeck, Germany

Addiction 2009 **104** (6) 921

Urinary ethyl glucuronide (EtG) and ethyl sulphate (EtS) assessment: Valuable tools to improve verification of abstention in alcohol-dependent patients during in-patient treatment and at follow-ups

The aims of this study were (i) to assess the effect of additional urinary ethyl glucuronide (EtG) and ethyl sulphate (EtS) assessment on diagnosed relapse rates in detoxified alcohol-dependent patients; and (ii) to compare dropout rates between EtG- and EtS-negative and -positive patients. Two studies on detoxified alcohol-dependent patients. If patients had no indication of relapse they were asked for a urinary sample at discharge from in-patient treatment 3, 6 and 12 weeks after discharge (study 1) and 1, 3 and 6 weeks after discharge (study 2), respectively. Department of Psychiatry, University of Luebeck, Germany. A total of 107 and 32 detoxified alcohol-dependent patients having participated in a 3-week in-patient motivation enhancement programme. Personal interviews, breathalyzer tests, assessment of urinary EtG and EtS with liquid chromatography-tandem mass spectrometry (LC-MS/MS analysis). Urinary EtG and EtS were always positive at the same time. In the first study 13.5% of the patients were already positive before being discharged from hospital. At the follow-ups 3, 6 and 12 weeks after discharge 12.2, 19.4 and 28.0%, respectively, of the patients coming to the follow-up and denying relapse were positive on urinary EtG and EtS. In the second study, of those patients showing up for follow-up after 1 week and denying relapse, EtG and EtS were positive in four cases (17.4%). Only one EtG- and EtS-positive relapser (3.1%) came to the next follow-ups. In both studies the rates of detected relapses were significantly higher for early follow-ups if urinary EtG and EtS results were considered additionally. Dropout rates until the next follow-up were significantly higher among positive than EtG- and EtS-negative patients. Urinary EtG and EtS improve verification of abstinence in studies of alcohol-dependent patients

Kerekes I, Yegles M*, Grimm U, Wennig R// *Univ Luxembourg, Lab Natl Sante-Toxicol, 162a Ave Faiencerie, LU-1511 Luxembourg, Luxembourg

Alcohol Alcohol 2009 **44** (1) 62

Ethyl glucuronide determination: Head hair *versus* non-head hair

Ethyl glucuronide (EtG), a non-volatile, water-soluble, direct metabolite of ethanol, in hair has previously been shown to be adequate for the detection of social and chronic excessive alcohol consumption. Scalp hair is not always available and analysis of hair from alternative anatomical sites becomes of interest. Hair samples from head, beard, chest, armpit, stomach, pubis, arms and legs from 32 subjects were analyzed in order to compare the EtG concentrations and to investigate whether the cut-offs used for head hair could be used for non-head hair. Following extraction by solid phase extraction using Oasis MAX columns and pentafluoropropionic anhydride (PFPA) derivatization, EtG was determined by GC/MS in negative chemical ionization mode using EtG-d5 as internal standard. In the cases of negative findings in head hair (EtG <7 pg/mg), in 7 out of 12 cases negative results were also be found in non-head hair. The five others were positive, due to a positive EtG finding in pubic hair. In 20 cases of positive EtG results for head hair, in all cases positive results could also be found in non-head hair. Although preliminary results indicate a clear trend regarding the concurrence between EtG results in head hair and non-head hair, interpretation of non-head hair results remains to be carefully done in the case of pubic hair, in which higher concentrations have often been found

Rowbottom L, Workman C, Roberts NB*// *Royal Liverpool & Broadgreen Univ Hosp, Dept Clin Biochem, 4th Floor, Duncan Bldg, Prescot St, Liverpool L7 8XP, England

Rapid Commun Mass Spectrom 2009 **23** (17) 2763

Evaluation of selected-ion flow-tube mass spectrometry for the measurement of ethanol, methanol and isopropanol in physiological fluids: Effect of osmolality and sample volume

Selected-ion flow-tube mass spectrometry (SIFT-MS) is particularly suited for the analysis of volatile low molecular weight compounds. We have evaluated this technique for the assay of different alcohols in aqueous solutions, including blood plasma, and in particular whether the osmolality or sample volume affected vapourisation. Solutions of three different alcohols (methanol, ethanol and isopropanol) ranging from 0.005 to 50 mmol/l were prepared in deionised water (0 milliosmol), phosphate-buffered saline (690 mOsm), isotonic saline (294 mOsm) and plasma (296 mOsm). The vapour above the sample (50 to 1000 µl) contained in air-tight tubes at 37°C was aspirated into the instrument. The outputs for ethanol, methanol and isopropanol were linear over the concentration range and independent of the sample volume and relatively independent of the osmolar concentration. SIFT-MS can reliably and accurately measure common alcohols in the headspace above aqueous solutions, including serum/plasma. This novel application of SIFT-MS is easy to follow, requires no sample preparation and the wide dynamic range will facilitate measurement of alcohols present from normal metabolism as well as when taken in excess or in accidental poisoning

14 Tobacco

Buszewski B, Ulanowska A, Ligor T, Denderz N, Amann A// Nicholas Copernicus Univ, Fac Chem, 7 Gagarin Str, PL-87100 Torun, Poland

Biomed Chromatogr 2009 **23** (5) 551

Analysis of exhaled breath from smokers, passive smokers and non-smokers by solid-phase microextraction gas chromatography/mass spectrometry

In this study, 38 samples of expired air were collected and analyzed from 20 non-smoking volunteers, four passive smokers and 14 smokers (21 women and 17 men). Measurements were carried out using solid-phase microextraction (SPME) as an isolation and preconcentration technique. The determination and identification were accomplished by gas chromatography coupled with mass spectrometry (GC/MS). Our data showed that *ca* 32% of all identified compounds in the breath of healthy non-smokers were saturated hydrocarbons. In the breath of smoking and passive smoking volunteers hydrocarbons were predominant, but also present were more exogenous analytes such as furan, acetonitrile and benzene than in the breath of non-smokers. Acetonitrile, furan, 3-methylfuran, 2,5-dimethylfuran, 2-butanone, octane and decane were identified in breath of smoking and passive smoking persons

Hazarika P, Jickells SM, Russell DA*// *Univ East Anglia, Sch Chem Sci & Pharm, Norwich NR4 7TJ, England

Analyst 2009 **134** (1) 93

Rapid detection of drug metabolites in latent fingermarks

Imaging of latent fingermarks through the detection of the cotinine antigen in the sweat deposited within the fingerprints of smokers has been achieved with magnetic particles functionalised with anti-cotinine antibody. The antibody-magnetic particle conjugates are easily applied to latent fingerprints and surplus reagents removed by means of a magnetic wand. Drug metabolites, such as cotinine, may be detected and used to image the fingermark to establish the identity of an individual within 15 minutes

Kim SR, Wipfli H, Avila-Tang E, Samet JM, Breysse PN// 615 Nth Wolf St, W7010H, Baltimore, Md 21205, USA

Biomed Chromatogr 2009 **23** (3) 273

Method validation for measurement of hair nicotine level in nonsmokers

The development of strategies to address the growing worldwide burden of exposure to secondhand smoke (SHS) would be facilitated by sensitive and accurate methods for assessing SHS exposure. Hair provides a readily available matrix for assessing biomarkers of typical SHS exposure. We developed and applied an optimized analytical method using an isotope dilution gas chromatography-mass spectrometry (GC/MS) for hair nicotine measurement. The utility of this optimized method is illustrated by presenting data on SHS exposure of women and children from 31 countries. Using this isotope dilution method with spiked samples (3.3 ng/mg), we found that the greatest hair nicotine extraction efficiency was obtained with a 60 min shaking time. In the field study (n = 2400), a positive association was evident between hair nicotine concentrations from nonsmokers and higher numbers of cigarettes smoked per day in a household

Kodama S, Morikawa A, Nakagomi K, Yamamoto A, Sato A, Suzuki K, Yamashita T, Kemmei T, Taga A// Toyama Inst Hlth, 17-1 Nakataikoyama, Kosugi, Toyama 939 0363, Japan

Electrophoresis 2009 **30** (2) 349

Enantioseparation of nicotine alkaloids in cigarettes by CE using sulfated -CD as a chiral selector and a capillary coated with amino groups

Nicotine (NC) and its related compounds (cotinine (CN), nornicotine (NN), anatabine (AT) and anabasine (AB)) were simultaneously enantioseparated by CE using a capillary with amino groups and sulfated β-CD as a chiral selector. The optimum running conditions were found to be 30 mM acetate buffer (pH 5.0) containing 8% sulfated β-CD with an applied voltage of +15 kV at 30°C using direct detection at 260 nm. Using a capillary coated with amino groups, the EOF migrates toward the positive pole. However, when sulfated β-CD was added to the BGE, it was found that the EOF migrated toward the negative pole due to ionic adsorption of sulfated β-CD to amino groups on the capillary inner wall. All the cationic analytes migrated as anions, suggesting that they formed stable anionic complexes with sulfated β-CD. With this system and a simple pretreatment with mini-cartridges, NC alkaloids in five cigarette samples were enantioseparated. As a result, each of the compounds except for CN was detected. In the case of NC, only (*S*)-NC was detected (more than 99.9%), but in the case of NN, AT and AB, the ratios of (*S*)-isomer to total isomers were in the ranges 58-70, 81-85 and 59-65%, respectively. On the other hand, only NC was detected in cigarette smoke and the ratio of (*S*)- and (*R*)-NCs was 96:4. The amounts of NC alkaloids in cigarettes suggest that the production of (*R*)-NC resulted from racemization due to the high temperature/burning of the cigarette

Koszowski B, Goniewicz ML, Czogala J, Zymelka A, Sobczak A// Med Univ Silesia, Fac Pharm & Lab Med, Dept Gen & Inorgan Chem, Sosnowiec, Poland

Int J Environ Anal Chem 2009 **89** (2) 105

Simultaneous determination of nicotine and 3-vinylpyridine in single cigarette tobacco smoke and in indoor air using direct extraction to solid phase

Two important markers of ETS exposure are nicotine and 3-vinylpyridine (3-ethenylpyridine, 3-EP). Their detection in mainstream (MS) and sidestream (SS) smoke of one single cigarette and in indoor air was achieved using direct solid phase extraction combined with gas chromatography. The technique may be employed for both nicotine and 3-EP determination in SS and MS of one single cigarette as well as it allows for a precise determination of compound distribution in indoor air. The method when employed for both kinds of samples allows anticipating indoor air distribution of both analysed compounds in a very precise way. The precision of the method (calculated as a relative standard deviation) was 9.78% for nicotine and 2.67% for 3-EP; whereas the accuracy (evaluated by a recovery study conducted at three different levels) was 70.1 and 87.3%, respectively. Limits of detection were 0.06 µg per cigarette for both nicotine and 3-EP. The compounds of interest in two commercially available brands of cigarettes as well as in the reference cigarettes 3R4F and also in indoor air polluted with tobacco smoke were determined to evaluate the technique. In MS, amounts varied from 586 to 772 (nicotine) µg per cigarette and from 3.5 to 10.7 (3-EP) µg per cigarette. In SS smoke the level varied from 14,370 to 22,590 (nicotine) µg per cigarette and from 185 to 550 (3-EP) µg per cigarette, whereas levels in indoor air polluted with tobacco smoke varied from 50.1 to 157.3 (nicotine) µg/m^3 and from 7.7 to 20.8 (3-EP) µg/m^3

Massadeh AM, Gharaibeh AA, Omari KW// Jordan Univ Sci & Technol, Fac Pharm, Dept Med Chem & Pharmacognosy, POB 3030, JO-22110 Irbid, Jordan

J Chromatogr Sci 2009 **47** (2) 170

A single-step, extraction method for the determination of nicotine and cotinine in Jordanian smokers' blood and urine samples by RP-HPLC and GC-MS

The determination of nicotine and cotinine in human plasma and urine in smokers has been achieved using a simple, rapid, reliable, and low cost one-step extraction method employing reversed-phase high-performance liquid chromatography (RP-HPLC) and gas chromatography-mass spectrometry (GC-MS). Run times were 16 and 10 min for HPLC and GC-MS, respectively. The method was validated over a wide linear range of 1-5000 ng/ml with correlation coefficients being consistently greater than 0.9985. Criteria considered for validation were: limit of quantitation, linearity, accuracy, precision, recovery, specificity, and selectivity. This study was conducted to evaluate the nicotine and cotinine content of Jordanian smokers' blood and urine samples; to study the relationship between the concentration of nicotine in urine and plasma samples; and to investigate the effect of pH on the extraction of nicotine and cotinine in urine samples. One hundred blood and urine samples are collected from eighty smokers and twenty nonsmokers. Samples were taken from volunteers after each had filled in a questionnaire. Nicotine concentrations in smokers' plasma were in the range of 181-3702 ng/ml with an average of 1263.1 ng/ml, in urine samples the range was 1364-1972 ng/ml, with an average of 1618 ng/ml. Cotinine concentrations in smokers' plasma were in the range of 21-4420 ng/ml with an average of 379.4 ng/ml, in urine the range was 6-3946 ng/ml with an average of 865 ng/ml. Statistical analysis indicated highly significant differences in nicotine and cotinine concentrations in smoker samples compared with nonsmoker samples ($p < 0.05$)

Wu CT, Chen PY, Chen JG, Suryanarayanan V, Ho KC*// *Natl Taiwan Univ, Dept Chem Engn, Taipei 10617, Taiwan

Anal Chim Acta 2009 **633** (1) 119

Detection of nicotine based on molecularly imprinted TiO_2-modified electrodes

A molecular imprinting technique with a titanium dioxide (TiO_2)/poly(3,4-ethylenedioxythiophene) (PEDOT)-modified electrode was employed for the amperometric detection of nicotine (NIC). PEDOT was coated onto the sintered electrode by *in situ* electrochemical polymerization of the monomer was utlized to improve the conductivity of the substrate. The sensing potential of the NIC-imprinted TiO_2 electrode (ITO/TiO_2[NIC]/PEDOT) in a phosphate-buffered saline (PBS) solution (pH 7.4) containing 0.1M KCl was determined to be 0.88 V (*vs*. Ag/AgCl/saturated KCl). The linear detection range for NIC oxidation on the so-called ITO/TiO_2[NIC]/PEDOT electrode was 0-5mM, with a sensitivity and limit of detection of 31.35 μA/mM/cm^2 and 4.9 μM, respectively. When comparing with the performance of the non-imprinted one, the sensitivity ratio was about 1.24. The increase in the electroactive area of the imprinted electrode resulted in higher sensitivity. The at-rest stability of the ITO/TiO_2[NIC]/PEDOT electrode was tested over a period of 3 days. At the end of 2 days, the current response remained about 85% of its initial value. The ITO/TiO_2[NIC]/PEDOT electrode showed reasonably good selectivity in distinguishing NIC from its major interferent, (-)-cotinine (COT). Furthermore, scanning electrochemical microscopy (SECM) was employed to elucidate the surface morphology of the imprinted and non-imprinted electrodes using $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ as a redox probe on a platinum tip. Further characterizion of the imprinted electrode was achieved by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR)

15 Homeland Security

Koskela H, Ervasti M, Bjork H, Vanninen P// Univ Helsinki, VERIFIN, POB 55, FI-00014 Helsinki, Finland

Anal Chem 2009 **81** (3) 1262

On-flow pulsed field gradient heteronuclear correlation spectrometry in off-line LC-SPE-NMR analysis of chemicals related to the chemical weapons convention

Complex samples may be analysed by hyphenation of liquid chromatography with nuclear magnetic resonance spectroscopy (LC-NMR). Unfortunately, application of on-flow ^{1}H NMR spectrometry during the LC-NMR analysis frequently suffers from high intensity of eluent resonances. Deuterated eluents or various signal suppression schemes may be used to improve the poor dynamic range. Deuterated eluents are expensive, and peak-selective signal suppression schemes are often unsatisfactory when detection of chemicals at low concentration is required. When the analytes have a common heteronucleus, on-flow pulsed field gradient heteronuclear correlation spectrometry may offer several benefits. Analytes may be monitored selectively, while the intense nondeuterated eluent and impurity background may be effectively eliminated. In the present study, on-flow one-dimensional (1D) ^{1}H-^{31}P heteronuclear single quantum coherence (HSQC) spectrometry was employed to analyse characteristic organophosphorus degradation products of nerve agents sarin and soman during chromatographic separation. These chemicals were not detectable by UV, so their retention times were monitored using on-flow 1D ^{1}H-^{31}P HSQC. This facilitated application of LC-NMR together with solid-phase extraction (LC-SPE-NMR) in analysis of these organophosphorus chemicals in an alkaline decontamination solution. The analytes were extracted from the SPE cartridges with deuterated eluent, and the off-line NMR analysis was achieved using a mass-sensitive microcoil probe head. The on-flow 1D ^{1}H-^{31}P HSQC approach presented a high dynamic range and good detection limit (ca. 10 μg/55 nmol) with a high sampling frequency (1 point per 2 s) in the acquired pseudo-two-dimensional spectrum. No significant impurity background was noted in the off-line NMR samples, and identification of the extracted analytes was straightforward

Lagarrigue M, Bossee A, Begos A, Delaunay N, Varenne A, Gareil P*, Bellier B// *Univ Paris 6, ENSCP, UMR 7575 CNRS, Lab Electrochim & Chim Anal, 11 rue Pierre & Marie Curie, FR-75231 Paris 05, France

Electrophoresis 2009 **30** (9) 1522

Analysis of nerve agent degradation products in high-conductivity matrices by transient ITP preconcentration and CZE separation coupled to ESI-MS

Preconcentration of nerve agent degradation products (alkyl methylphosphonic acids) contained in high-conductivity matrices was performed using transient ITP to enhance sensitivity of CE-ESI-MS. The separation conditions of the five studied alkyl methylphosphonic acids in CE-MS were first optimized. The presence of methanol in the separation medium was required to obtain a good separation of the analytes under counter-EOF conditions. Preconcentration by ITP was induced by the BGE acting as leading electrolyte (LE) while the terminating electrolyte (TE) was loaded before the sample because of the counter-EOF conditions. Different leading ions (formate or acetate) and LE concentrations were tested. The best results for the analysis of soil extracts fortified with the analytes were obtained with an LE composed of 30 mM CH_3COONH_4 adjusted to pH 8.8 with ammonium hydroxide in (35:65 v/v) MeOH/H_2O mixture. The TE consisted of 200 mM glycine adjusted to pH 10.0 with ammonium hydroxide in the same solvent mixture. The loading length of the TE zone was optimized. The initial pH of the TE, which determined the initial mobility of the terminating ion, appeared to markedly influence the resolution and the sensitivity. This transient ITP-CZE-MS method was then adapted for the analysis of rat urine samples fortified with the analytes, which required the use of a more concentrated LE (50 mM). LODs between 4 and 70 ng/ml in soil extract, and between 5 and 75 ng/ml in rat urine were reached from extracted ion electropherograms

Pal S, Alocilja EC*// *Michigan State Univ, Dept Agric & Biosystem Engn, 204 Farrall Hall, East Lansing, Mi 48824, USA

Biosens Bioelectron 2009 **24** (5) 1437

Electrically active polyaniline coated magnetic (EAPM) nanoparticle as novel transducer in biosensor for detection of *Bacillus anthracis* spores in food samples

The detection of *Bacillus anthracis* endospores in contaminated food samples was achieved by an electrically active polyaniline coated magnetic (EAPM) nanoparticle-based biosensor. The 100 nm-diameter EAPM nanoparticles were produced from aniline monomer (made electrically active by acid doping) coating the surface of γ iron oxide cores. EAPM nanoparticles have been studied using superconducting quantum interference device (SQUID), four-point probe, and transmission electron microscopy (TEM) in respect of magnetic, electrical, and structural characteristics. Ambient temperature hysteresis of the synthesized nanoparticles showed a saturation magnetization value of 44.1 emu/g. The EAPM nanoparticles were biologically modified to act as an immunomagnetic concentrator of B. anthracis spores from lettuce, ground beef and whole milk samples and are directly applied to a direct-charge transfer biosensor. The detection mechanism of the biosensor depended upon the capillary flow of the captured spores on the biosensor surface along with direct-charge transfer across the EAPM nanoparticles. The biosensor was capable of detecting *B. anthracis* spores at concentrations as low as 4.2 x 10^2 spores/ml from the samples. The EAPM-based biosensor is fast and reliable with a total detection time of 16 min

Prathish KP, Vishnuvardhan V, Rao TP*// *CSIR, NIIST, Trivandrum, Kerala, India

Electroanalysis 2009 **21** (9) 1048

Rational design of *in situ* monolithic imprinted polymer membranes for the potentiometric sensing of diethyl chlorophosphate - A chemical warfare agent simulant

Molecularly imprinted polymer membrane was prepared by semicovalent imprinting strategy wherein i) the template diethyl chlorophosphate (DCP), (a simulant of organophosphorous nerve agents), is covalently linked to the reactive functional monomer vinyl aniline (VA) during imprinting step followed by

noncovalent rebinding and ii) *in situ* polymerization *via* single pot synthesis in presence of additional functional monomer, 2-hydroxyethyl methacrylate (HEMA) and crosslinking monomer, ethylene glycol dimethacrylate (EGDMA) after addition of 2-nitrophenyl octyl ether (NPOE) and 2,2-azobisisobutyronitrile (AIBN) as plasticizer and initiator respectively. The resulting membrane is integrated with a potentiometric transducer while designing a DCP sensor. The fabricated sensor responds over a wider concentration range of 10^{-6}-10^{-2} M with a lower detection limit of 10^{-6} M (0.17 ppm). In addition, *in situ* monolithic membrane based sensor was designed by adopting noncovalent imprinting strategy also. A detailed comparison is made between semicovalent and noncovalent *in situ* membrane based sensors on the prime sensor performance criteria such as sensitivity, selectivity, working range, response time, reusability and reversibility. Again, the relative merits and demerits of semicovalent *vis-à-vis* noncovalent strategy based *in situ* monolithic membrane sensors were also highlighted. The probable molecular recognition mechanism is also discussed

Seiman A, Jaanus M, Vaher M, Kaljurand M// Tallinn Univ Technol, Dept Chem, Akad Tee 15, EE-12618 Tallinn, Estonia
Electrophoresis 2009 **30** (3) 507
A portable capillary electropherograph equipped with a cross-sampler and a countactless-conductivity detector for the detection of the degradation products of chemical warfare agents in soil extracts
A fully portable CE device equipped with a capacitively coupled contactless-conductivity detector and a cross-injection device is put to the test in laboratory conditions. The portable device is capable of working on batteries for at least 4 h. After that, its performance is strongly affected by the drop in the high-voltage output and analysis may be interrupted if its length exceeds a reasonable time. The concentration of the BGE affects both ionic strength and conductivity. Choosing an optimal concentration of BGE is therefore about finding a good compromise between selectivity and sensitivity. All experiments were performed using a mixture of histidine and MES with a concentration of 15 mM as BGE. The performance of the cross-injection device is optimized by the use of internal standards. Satisfactory reproducibility is gained as the RSD of peak areas is reduced to 8% or less. LODs for different phosphonic acids are in the range of 2.5-9.7 µM. For the analysis of adsorption of phosphonic acids in sand and loamy soil samples, calibration curves are constructed. Linearity in a measured concentration range of 10-100 µM is excellent, as the squares of correlation constants are approximately 1. The concentration analysis of phosphonic acids in soil extracts demonstrates that their adsorption curves in sand and loamy soil follow different adsorption isotherms

16 Workplace

Campo L, Mercadante R, Rossella F, Fustinoni S// Via Barnaba 8, IT-20122 Milan, Italy
Anal Chim Acta 2009 **631** (2) 196
Quantification of 13 priority polycyclic aromatic hydrocarbons in human urine by headspace solid-phase microextraction gas chromatography-isotope dilution mass spectrometry
In both living and working environments, polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants. This study was conducted to develop a headspace solid-phase microextraction gas chromatography-isotope dilution mass spectrometry (HS-SPME/GC-IDMS) method for the simultaneous quantification of 13 PAHs in urine samples. Conditions influencing PAHs extraction by HS-SPME were considered and optimized, for example, type/thickness of fiber coatings, extraction temperature/time, desorption temperature/time, ionic strength and sample agitation. The stability of spiked PAHs solutions and of real urine samples stored up to 90 days in containers of different materials was investigated. For the optimized method, analytes were absorbed for 60 min at 80ºC in the sample headspace with a 100µm polydimethylsiloxane fiber. The method is very specific, with linear range from the limit of quantification to 8.67 x 10^3 ng/l, a within-run precision of <20% and a between-run precision of <20% for 2-, 3- and 4-ring compounds and of <30% for 5-ring compounds, trueness within 20% of the spiked concentration, and limit of quantification in the 2.28-2.28 x 10^1 ng/l range. The wa applied to 15 urine samples from subjects exposed to PAHs at different environmental levels

Hernandez F, Portoles T, Pitarch E, Lopez FJ// Univ Jaume I, Res Inst Pesticides & Water, ES-12071 Castellon, Spain
J Mass Spectrom 2009 **44** (1) 1
Special feature: Perspective - Searching for anthropogenic contaminants in human breast adipose tissues using gas chromatography-time-of-flight mass spectrometry
The potential of gas chromatography-time-of-flight mass spectrometry (GC-TOF MS) for screening anthropogenic organic contaminants in human breast adipose tissues has been investigated. Initially a target screening was performed for a list of 125 compounds which included persistent halogen pollutants [organochlorine (OC) pesticides, polychlorinated biphenylss (PCBs), polybrominated diphenyl ethers (PBDEs)], polyaromatic hydrocarbons (PAHs), alkylphenols, and a notable number of pesticides from the different fungicide, herbicide and insecticide families. Searching for target pollutants was done by evaluating the presence of up to five representative ions for every analyte, all measured at accurate mass (20-mDa mass window). The experimental ion abundance ratios were then compared to those of reference standards for confirmation. Sample treatment consisted of an extraction with hexane and subsequent normal-phase (NP) high performance liquid chromatography (HPLC) or SPE cleanup. The fat-free LC fractions were then investigated by GC-TOF MS. Full-spectral acquisition and accurate mass data generated by GC-TOF MS also allowed the investigation of nontarget compounds using appropriate processing software to manage MS data. Identification was initially based on library fit using commercial nominal mass libraries. This was followed by comparing the experimental accurate masses of the most relevant ions with the theoretical exact masses with calculations made using the elemental composition calculator included in the software. The application of both target and nontarget approaches to around 40 real samples allowed the detection and confirmation of several target pollutants including *p*,*p*'-DDE, hexachlorobenzene (HCB), and some polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs). Several nontarget compounds that could be considered anthropogenic pollutants were also detected. These included 3,5-di-*tert*-butyl-4-hydroxy-toluene (BHT) and its metabolite 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde (BHT-CHO), dibenzylamine, *N*-butyl benzenesulfonamide (*N*-BBSA), some naphthalene-related compounds and several PCBs isomers not included in the target list. As some of the compounds detected are xenoestrogens, the methodology developed in this paper could be useful in human breast cancer research

Liu Y, Muralidhara S, Bruckner JV, Bartlett MG*// *Univ Georgia, Coll Pharm, Dept Pharmaceut & Biomed Sci, Athens, Ga 30602, USA
Rapid Commun Mass Spectrom 2009 **23** (3) 455
Determination of trichloroethylene from adipose tissue by headspace solid-phase microextraction gas chromatography/mass spectrometry
No abstract available

Wang VS, Lu MY// China Med Univ, Dept Occupat Safety & Hlth, 91 Hsueh Shih Rd, Taichung 404, Taiwan
J Chromatogr B 2009 **877** (1-2) 24
Application of solid-phase microextraction and gas chromatography-mass spectrometry for measuring chemicals in saliva of synthetic leather workers
Testing saliva is noninvasive and less confidential in comparison with blood and urine. It may be employed to detect illicit drug abuse and for monitoring of exposure to hazardous solvents. Headspace solid-phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry (GC-MS), was employed by which the saliva matrix may be monitored for multiple compounds with various polarities, such as methyl ethyl ketone (MEK), isopropyl alcohol (IPA), and *N*,*N*-dimethyl formamide (DMF) (common solvents used in synthetic leather manufacture), as well as acetone (ACE) and *N*-methyl formamide (NMF) (metabolites of IPA and DMF, respectively). Carboxen/polydimethylsiloxane (CAR/PDMS 75 µm) fiber coating was employed and various extraction and desorption parameters were investigated. Extraction efficiency and reproducibility of analyses was improved by pre-incubation. Limits of detection were 0.004, 0.003, 0.006, 0.05, and 0.10 µg/ml for ACE, MEK, IPA, DMF, and NMF, respectively. Validation was achieved with standards spiked in blank saliva, and a correlation was made between HS-SPME and traditional solvent pretreatment methods. It was found that correlation coefficients (r) were greater than 0.996 for each analyte, with no significant differences ($p > 0.05$) between two methods. The SPME method resulted in lower limits of detection, with good accuracy (recovery 95.3-109.2%) and precision (1.17-8.22% CV) for both intra- and inter-assay, when quality control samples were analyzed for all five compounds. The partition coefficient for each compound between the headspace of the saliva sample and the CAR/PDMS fiber coating was 90.9, 170.1, 36.4, 3.70 and 0.92 for ACE, MEK, IPA, DMF and NMF, respectively. Saliva from workers in a synthetic leather factory were investigated. The SPME method is a highly versatile and flexible technique for chemical measurement, and its application for monitoring biological exposure to hazardous solvents is evident. Using sensitive SPME approaches, analysis of saliva for determining workplace exposure should prove useful as an alternative exposure monitoring method

17 Product Authenticity

Ahn S, Hong JY, Hong MK, Jang YP, Oh MS, Jung JH, Hong J*// *Kyung Hee Univ, Coll Pharm, Hoegi-dong, Dongdaemoon-ku, Seoul 130 701, South Korea

Rapid Commun Mass Spectrom 2009 **23** (19) 3158

Structural determination of sildenafil and its analogues in dietary supplements by fast-atom bombardment collison-induced dissociation tandem mass spectrometry

Sildenafil and its analogues, which are used as illegal additives in several dietary supplements, were isolated by liquid-liquid extraction and column chromatography and analyzed by fast-atom bombardment mass spectrometry (FAB-MS). Structures of sildenafil and its derivatives were elucidated by FAB-tandem mass spectrometry (MS/MS) with exact mass measurement in the positive-ion mode. To find structurally diagnostic ions for the sildenafil analogues, authentic sildenafil was preferentially analyzed by high-energy collision-induced dissociation (CID)-MS/MS. The CID-MS/MS spectra of $[M+H]^+$ precursor ions resulted in the formation of numerous characteristic ions *via* a series of dissociative processes. The product ions formed by CID provided important information on the modification of the piperazine ring, the phenylsulfonyl group and the pyrazolopyrimidine moiety of sildenafil. By interpreting their MS/MS spectra, the chemical structures of sildenafil analogues isolated from dietary supplements could be elucidated and fragmentation patterns were proposed. To clearly identify the sidenafil derivatives in dietary supplements, some of the derivatives such as acetildenafil, homosildenafil and hydroxyhomosildenafil which are not commercially available were synthesized and compared with their MS/MS spectra. In addition, high-resolution mass measurements were conducted to obtain the elemental compositions of sildenafil and its analogues

18 Techniques

Bazylak G, Monge ME, Everaert J, Nagels LJ// Nicholas Copernicus Univ, Fac Pharm, Coll Med, Dept Pharmaco-Bromatol & Mol Nutr, Jagiellonska 13, PL-85067 Bydgoszcz, Poland

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Hydrophobicity-aided potentiometric detection of catecholamines, -agonists and -blockers, in a mixed-solvent capillary electrophoresis system

A series of cationic drug-like substances with distinct basicity, hydrogen-bonding ability, and hydrophobicity, including three catecholamines, two β-agonists, and thirteen β-blockers, was successfully detected in a capillary electrophoresis system using an end-capillary coupled potentiometric sensor consisting of a PVC-based liquid membrane deposited directly on a 100 µm diameter copper rod. The electrophoretic separation was performed on a 72 cm x 75 µm id uncoated fused-silica capillary with an acidic background electrolyte containing phosphoric acid in a water-acetonitrile mixture, pH* 2.8. Samples were injected electrokinetically at 5.0 kV for 10 s and a running voltage of 19.5 kV was applied. Excluding the bufuralol/practolol pair, baseline separation of all substances was achieved in the developed CE system within 9 minutes. A linear relationship (r^2 0.8752) between the sensitivity of the applied potentiometric detector and the parameter log P characterising the hydrophobicity of the analytes was demonstrated. The best observable limits of detection (LODs) were obtained for the highly hydrophobic substances, i. e. bufuralol (8.10 x 10^{-8} M injected concentration, *S/N* = 3), propranolol, alprenolol, and clenbuterol (ca. 1.10 x 10^{-7} M). In the case of hydrophilic catecholamines and carbuterol their LODs with potentiometric detection were lowered by a factor of almost one thousand, reaching a value of 6.6 x 10^{-5} M

Cho SH, Lee J, Choi MH, Lee WY, Chung BC*// *KIST, Bioanal & Biotransformation Res Ctr, 39-1 Hawolkok dong, Sungbuk gu, Seoul 136 791, South Korea

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Determination of urinary androgen glucuronides by capillary electrophoresis with electrospray tandem mass spectrometry

Capillary electrophoresis-electrospray tandem mass spectrometry (CE-ESI/MS/MS) is a simple and highly sensitive method for quantifying seven urinary androgen glucuronides. The urine samples were diluted and filtered through a membrane filter, and the filtrate was injected into a CE-MS/MS system without further sample preparation steps such as extraction and derivatization. The calibration ranges were 0.01-5 µg/ml for glucuronides of androsterone and 11β-OHAn-3G, and 5-500 ng/ml for glucuronides of 11-ketoAn, DHEA, testosterone, epitestosterone and DHT. The linearity of the method was 0.992-0.998, and the limits-of-detection at a signal-to-noise ratio of 3 were 5-10 ng/ml. The coefficients of variation were in the range of 4.0-9.0% for intra-day assay and 4.1-9.8% for inter-day assay. The proposed method may be applicable to metabolic profiling in both quantitative and qualitative analysis

Fountain KJ, Yin Z, Diehl DM// Waters Corp, Chem Appl Technol, 34 Maple St, Milford, Ma 01757, USA

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Simultaneous analysis of morphine-related compounds in plasma using mixed-mode solid phase extraction and ultra performance liquid chromatography-mass spectrometry

A bioanalytical method using mixed-mode solid phase extraction and UltraPerformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was developed for the analysis of morphine, morphine-3ß-glucuronide, morphine-6ß-glucuronide, 6-acetylmorphine, morphine *N*-oxide, and 10-hydroxymorphine in porcine plasma. All six compounds, along with four deuterated internal standards, were simultaneously extracted using mixed-mode strong cation exchange SPE in a 96-well µElution plate format. Due to analyte instability, a neutralizing solvent was used during the elution step to minimize degradation of 6-acetylmorphine. Separation was subsequently performed in 8 minutes on a 2.1 x 100 mm, 1.8 µm C_{18} column designed for retention of extremely polar compounds using a formic acid and methanol gradient. Analytes were detected by positive electrospray ionization in multiple reaction monitoring mode using a fast-scanning triple quadrupole mass spectrometer. Recovery was 73-123% depending on the analyte, and inter-day variability was less than 6%. Linearity was determined in porcine plasma by spiking the analytes prior to SPE. Correlation coefficients were >/=0.998, and % deviation from the actual concentrations was less than 15%. The lower limit of quantitation (LLOQ) for all compounds was between 0.1 and 0.25 ng/ml

Fox EJ, Twigger S, Allen KR// Leeds Teaching Hosp NHS Trust, Dept Clin Biochem, Britannia House, Leeds LS27 0DQ, England

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Criteria for opiate identification using liquid chromatography linked to tandem mass spectrometry: Problems in routine practice

Whereas no official standards exist for drug identification using liquid chromatography linked to tandem mass spectrometry (LC/MS/MS), the European Union (EU) criteria for compound identification have been adopted. These criteria have been assessed in respect of opiate confirmation by LC/MS/MS andissues highlighted. Opiate-positive urine samples tested with immunoassay were subjected to confirmation by LC/MS/MS using multiple reaction monitoring (MRM) and two separate buffer systems of pH 6.8 and 8.0, respectively. EU criteria for compound identification were applied for confirmation of morphine, 6-monoacetylmorphine (6MAM), codeine and dihydrocodeine (DHC). Employing the pH 6.8 buffer, confirmation could be achieved for 84%, 94%, 96% and 95%, respectively, for samples demonstrating MRM chromatographic peaks at retention times for morphine, 6MAM, codeine and DHC. Inability to meet the EU criteria was primarily attributed to low signal-to-noise (S:N) ratios or excessively high drug concentrations. Isobaric interferences and poor chromatography also contributed. Identification of morphine was greatly improved using chromatography at pH 8.0 owing to resolution of interferences. Oxycodone metabolites were a potential problem for the identification of DHC. Isobaric interferences may pose a problem with drug identification using LC/MS/MS. Optimizing chromatographic conditions is necessary to mitigate these interferences. Consideration should also to be given to investigating drug metabolites as well as parent drugs in method development

Guthery B, Bassindale A, Pillinger CT, Morgan GH// Open Univ, Planetary & Space Sci Res Inst, Milton Keynes MK7 6AA, England

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The detection of various opiates and benzodiazepines by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry

A technique using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry (GC x GC/TOFMS) is applied to qualitative and quantitative drug testing. Human serum was 'spiked' with known quantities of benzodiazepines and a 'street heroin' mixture including some of the major metabolites and impurities. The sample components were extracted from the matrix by solid-phase extraction (SPE). Constituents containing polar hydroxyl and/or secondary amine groups were derivatised with *N*-methyl-*N*-(*tert*-butyldimethyl)trifluoroacetamide (MTBSTFA) to improve the chromatographic performance. An orthogonal separation of the matrix constituents was achieved by coupling a DB-5ms (5% phenyl) to a BPX50 (50% phenyl) GC column. The eluant was focused onto the second column by a twin-stage cryo-modulator. Rapid 6 s modulation times were achieved by transfer from a 30 m x 0.25 mm (length x internal diameter) to a 2 m x 0.1 mm column. TOFMS with rapid spectral acquisition (< or = 500 spectra/s) was employed in the mass range *m/z* 40-650. A clean mass spectrum was obtained for each analyte using mass spectral deconvolution software. The sensitivity and repeatability of the method were evaluated by the preparation of calibration standards for two benzodiazepines, flunitrazepam and its major metabolite 7-aminoflunitrazepam (7-amino-FN), in the concentration range 5-1000 ng/ml. The limits of detection (LODs) and limits of quantitation (LOQs), calculated by repeat injections (x10) of the lowest standard, were 1.6 and 5.4 ng/ml (flunitrazepam); 2.5 and 8.5 ng/ml (7-amino-FN), respectively. There is scope to extend this protocol to screen a large number of drugs and metabolites stored in a library database

Liao QF, Deng YT, Xie ZY*, Pan BY, Zhang L// *Sun Yat Sen Univ, Sch Pharmaceut Sci, Lab Pharmaceut Anal & Qual Assessment, CN-510080 Guangzhou, Guangdong, Peoples Rep China

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Rapid simultaneous determination of codeine and morphine in plasma using LC-ESI-MS/MS: Application to a clinical pharmacokinetic study

A rapid and sensitive high-performance LC-MS/MS method was developed and validated for the simultaneous quantification of codeine and its metabolite morphine in human plasma using donepezil as an internal standard (IS). Following a single liquid-liquid extraction with ethyl acetate, the analytes were separated using an isocratic mobile phase on a C_{18} column and analyzed by MS/MS in the selected reaction monitoring mode using the respective $[M+H]^+$ ions, mass-to-charge ratio (*m/z*) 300/165 for codeine, *m/z* 286/165 for morphine and *m/z* 380/91 for IS. The method exhibited a linear dynamic range of 0.2-100/0.5-250 ng/ml for codeine/morphine in human plasma, respectively. The lower LOQs were 0.2 and 0.5 ng/ml for codeine and its metabolite morphine using 0.5 ml of human plasma. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. A run time of 2.0 min for each sample made it possible to analyze more than 300 human plasma samples per day. The validated LC-MS/MS method was applied to a pharmacokinetic study in which healthy Chinese volunteers each received a single oral dose of 30 mg codeine phosphate

Nakamura M, Ohmori T, Itoh Y, Terashita M, Hirano K// Gifu Pharmaceut Univ, Lab Drug Informat, 5-6-1 Mitahora Higashi, Gifu 502, Japan

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Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography-tandem mass spectrometry using a high-resolution octadecyl silica column compatible with aqueous compounds

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method using a high-resolution octadecyl silica column compatible with aqueous compounds was developed for the simultaneous determination of benzodiazepines and their metabolites in human serum. This method enabled us to determine multiple benzodiazepines, including flurazepam, bromazepam, chlordiazepoxide, nitrazepam, clonazepam, flunitrazepam, estazolam, clobazam, lorazepam, alprazolam, triazolam, brotizolam, fludiazepam, diazepam, quazepam, prazepam and their metabolites such as 7-aminonitrazepam, 7-aminoclonazepam, 7-acetamidonitrazepam, *N*-desmethylclobazam and *N*-desmethyldiazepam. The analytes spiked into human serum were subjected to solid-phase extraction followed by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. The running time was within 25 min for the measurement of 22 benzodiazepines and their metabolites. The recovery rates exceeded 58.1% for those compounds except for quazepam, which showed a recovery of 45.8%. The limit of detection ranged from 0.3 to 11.4 ng/ml. Linearity was satisfactory for all compounds. These data suggest that the present method can be applicable to routine assay for benzodiazepines in the clinical setting

Pelander A, Tyrkko E, Ojanpera I// Univ Helsinki, Dept Forensic Med, PO Box 40, FI-00014 Helsinki, Finland

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***In silico* methods for predicting metabolism and mass fragmentation applied to quetiapine in liquid chromatography/time-of-flight mass spectrometry urine drug screening**

Current *in silico* tools were evaluated for their ability to predict metabolism and mass spectral fragmentation in the context of analytical toxicology practice. A metabolite prediction program (Lhasa Meteor), a metabolite detection program (Bruker MetaboliteDetect), and a fragmentation prediction program (ACD/MS Fragmenter) were used to assign phase I metabolites of the antipsychotic drug quetiapine in the liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS) accurate mass data from ten autopsy urine samples. In the literature, the main metabolic routes of quetiapine have been reported to be sulfoxidation, oxidation to the corresponding carboxylic acid, *N*- and *O*-dealkylation and hydroxylation. Of the 14 metabolites predicted by Meteor, eight were detected by LC/TOFMS in the urine samples with use of MetaboliteDetect software and manual inspection. An additional five hydroxy derivatives were detected, but not predicted by Meteor. The fragment structures provided by ACD/MS Fragmenter software confirmed the identification of the metabolites. Mean mass accuracy and isotopic pattern match (SigmaFit) values for the fragments were 2.40 ppm (0.62 mDa) and 0.010, respectively. ACD/MS Fragmenter, in particular, allowed metabolites with identical molecular formulae to be differentiated without a need to access the respective reference standards or reference spectra. This was well exemplified with the hydroxy/sulfoxy metabolites of quetiapine and their *N*- and *O*-dealkylated forms. The procedure resulted in assigning 13 quetiapine metabolites in urine. The present approach is instrumental in developing an extensive database containing exact monoisotopic masses and verified retention times of drugs and their urinary metabolites for LC/TOFMS drug screening

Pozo OJ, Ventura R, Monfort N, Segura J, Delbeke FT// UGent, Dept Clin Chem Microbiol & Immunol, DoCoLab, Technologiepark 30, BE-9052 Zwijnaarde, Belgium

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Evaluation of different scan methods for the urinary detection of corticosteroid metabolites by liquid chromatography tandem mass spectrometry

Different approaches for the non-target detection of corticosteroids in urine have been evaluated. As a result of previous studies about the ionization (positive/negative) and fragmentation of corticosteroids, several methods based on both precursor ion (PI) and neutral loss (NL) scans are proposed. The applicability of these methods was checked by the injection of a standard solution containing 19 model compounds. Five of the studied methods (NL of 76 Da; PI of 77, 91 and 105; PI of 237; PI of 121, 147 and 171; and NL of 38 Da) exhibited satisfactory results at the concentration level checked (corresponding to 20 ng/ml in sample). Some other methods in negative ionization mode such as the NL of 104 Da were found to lack sufficient sensitivity. Some of the applied methods were found to be specific for a concrete structure (NL of 38 Da for fluorine containing corticosteroids) while others showed a wide range applicability (PI of 77, 91 and 105 showed response in all model compounds). Interference by endogenous compounds was also tested by the analysis of negative urines and urines spiked with different corticosteroids. The suitability of these methods for the detection of corticosteroid metabolites was checked by the analysis of urine samples collected after the administration of methylprednisolone and triamcinolone. A combination of the reported methods seems to be the approach of choice in order to have a global overview about the excreted corticosteroid metabolites

Vissers B, Everaert J, Sekula J, Malak A, Bohets H, Bazylak G*, Nagels LJ// *Nicholas Copernicus Univ, Fac Pharm, Coll Med, Dept Pharmaco-Bromatol & Mol Nutr, Jagiellonska 13, PL-85067 Bydgoszcz, Poland

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Unique potentiometric detection systems for HPLC determination of some steroids in human urine

Isocratic HPLC with potentiometric detection is used for the determination of some 17-ketosteroids (17-KS), e.g., androsterone, dehydroepiandrosterone and estrone, and their respective sulfated conjugates (17-KSS). Glassy carbon or composite electrodes containing a mixture of graphite and poly(vinyl chloride), PVC, were used as substrate electrodes. These substrates were covered either by montmorillonite or potassium tetrakis(*p*-chlorophenyl) borate containing PVC-based rubber phase membranes. The neutral 17-KS compounds were derivatized with Girard's reagent P (GP) to obtain cationic pyridinium acetohydrazones prior to the HPLC/potentiometric detection assay. No side reactions were observed, and the GP itself was not interfering. The method yielded accurate and reproducible results and was applicable to samples containing down to micromolar concentrations. Next, the 17-KSS compounds, acting as anionic charged molecules, were determined directly in human urine samples with the HPLC/potentiometry combination without preliminary derivatization. For this purpose, a new anion-sensitive potentiometric electrode was developed using a macrocyclic polyamine containing, PVC-based, rubber phase membrane. The three 17-KSS compounds were also determined accurately down to micromolar concentrations. Especially, the main androgen metabolites as dehydroepiandrosterone sulfate and androsterone sulfate could be selectively determined with a developed potentiometric sensor in human urine samples without time-consuming cleanup and preconcentration step

Zheng MM, Zhang MY, Feng YQ*// *Wuhan Univ, Dept Chem, CN-430072 Wuhan, Peoples Rep China

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Polymer monolith microextraction online coupled to hydrophilic interaction chromatography/mass spectrometry for analysis of $_2$-agonist in human urine

An analytical method based on online combination of polymer monolith microextraction (PMME) technique with hydrophilic interaction LC (HILIC)/MS is presented. The extraction was performed with a poly(methacrylic acid-*co*-ethylene glycol dimethacrylate) monolithic column while the subsequent separation was carried out on a Luna silica column by HILIC. After 1:1 v/v dilution with 20 mM phosphate solution at pH 7.0 and centrifugation, urine sample was directly used for extraction. After optimization, 85% ACN (containing 0.3% formic acid v/v) was used for rapid online elution, which was also the mobile phase in HILIC to avoid band broadening during separation or carry-over that was usually observed in PMME-RP LC system. Online automation of extraction and separation procedures was realized under the control of a program in this study. The developed method was applied to rapid and sensitive monitoring of three β_2-agonist traces in human urine. The LODs ($S/N = 3$) of the method were found to be 0.05-0.09 ng/ml of β_2-agonists in urine. The recoveries of three β_2-agonists spiked in five different urine samples ranged from 79.8 to 119.8%, with RSDs less than 18.0%