Published online in Wiley Online Library 2010

(wileyonlinelibrary.com) DOI:10.1002/dta.65

Current awareness in drug testing and analysis

1 Reviews

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Trends Anal Chem 2010 **29** (3) 209
Analytical procedures for determination of cocaine and its metabolites in biological samples
The deleterious effects and growth in usage of illicit drugs have stimulated the development of many analytical techniques. This review of analytical methods for the determination of cocaine (COC) and its metabolites is based upon the literature from the past two decades. The primary objective is to compare the capabilities of the different analytical protocols. The diversity of matrices from which COC is analyzed (e.g., blood, hair, saliva, plasma, urine, inner organs and meconium) has resulted in differences in sample preparation. To adequately investigate drug exposure, it is necessary to select the appropriate sample type and the analytical technique. Relevant sampling, extraction and purification techniques are discussed

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Trends Anal Chem 2010 **29** (3) 246
Analysis of meconium, nails and tears for determination of medicines and drugs of abuse
This review describes techniques for the analysis of medicines and drugs of abuse in three biological specimens – meconium, nails and tears – based upon the literature published since 1998. It commences with general descriptions of specimens, sample-collection methods and sample-preparation protocols. Crucial issues discussed relate to drug determination in meconium, nails and tears

2 Sports Doping - General

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Int J Sports Med 2010 **31** (2) 75
Red blood cell-mimicking synthetic biomaterial particles: The new frontier of blood doping? (Invited Editorial)
No abstract available

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Anal Bioanal Chem 2010 **396** (7) 2583
Generic sample preparation combined with high-resolution liquid chromatography-time-of-flight mass spectrometry for unification of urine screening in doping-control laboratories
A unification of doping-control screening procedures of prohibited small molecule substances (including stimulants, narcotics, steroids, β2-agonists and diuretics) is crucial in order to release resources for new classes such as banned proteins. Notionally, this might be accomplished by the use of a combination of one gas chromatography-time-of-flight mass spectrometry method and one liquid chromatography-time-of-flight mass spectrometry method. A quantitative screening technique using high-resolution liquid chromatography in combination with accurate-mass time-of-flight mass spectrometry has been developed and validated for the determination of glucocorticosteroids, β2-agonists, thiazide diuretics, and narcotics and stimulants in urine. To facilitate the simultaneous isolation of all compounds of interest and the required purification of the resulting extracts, a generic extraction and hydrolysis procedure was combined with a solid-phase extraction modified for these groups of compounds. Most compounds were determined using positive electrospray ionisation. However, for thiazide diuretics, the best sensitivity was obtained by employing negative electrospray ionisation. Data illustrate that with the exception of clenhexyl, procaterol, and reproterol, all compounds can be detected below the respective minimum required performance level. In addition, linearity, repeatability, within-lab reproducibility, and accuracy show that the method may be employed for quantitative screening. Should qualitative screening be sufficient, instrumental analysis may be limited to positive ionisation because all analytes (including thiazides) may be detected at the respective minimum required levels in the positive mode. The application of accurate-mass time-of-flight mass spectrometry in combination with generic extraction and purification procedures has proved applicable for the unification and expansion of the window of screening methods of doping laboratories. Furthermore, full-scan accurate-mass data sets will allow retrospective examination for emerging doping agents, without re-analyzing the samples

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Int J Sports Med 2010 **31** (1) 5
Desmopressin and hemodilution: Implications in doping
Blood doping enhances physical performance in sport. Plasma volume expanders/antidiuretics are banned agents employed to mask an artificial increase in hematological values resulting from blood doping. The chosen antidiuretic was desmopressin (DDAVP). This works examines whether DDAVP-induced hemodilution alters the concentration of hematological parameters employed to detect blood doping. This was an intra-subject crossover study. Venous blood samples were acquired from eight physically active males on two occasions. In the first instance, subjects ingested 1.5 litres of mineral water and 4.3 μg/kg of DDAVP whereas on the second, the subjects imbibed simply 1.5 litres of mineral water. Samples were analyzed for hematocrit, hemoglobin, reticulocytes, OFF Hr-Score, glucose, albumin, creatinine and total proteins. Following DDAVP, there was a significant decrease in the hematocrit, hemoglobin and in the OFF Hr-Score values. In addition, there was a significant decrease in glucose, albumin, creatinine and total proteins concentration. However, in this

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.

instance, all the values were significantly below the physiological levels. Therefore, administration of DDAVP is a very effective hemodilution effect. It is proposed that DDAVP should be included in the WADA's prohibited list

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Drug Alcohol Depend 2010 **106** (2) 230
Randomized response estimates for doping and illicit drug use in elite athletes
Presently, the percentage of unknown cases of doping and illicit drug use in fitness sports has been estimated. However, that is not the case for elite sports. This may result from the issue of implementing questionnaires and surveys to obtain reliable epidemiological estimates of deviant or illicit behaviour. Athletes addressed were subject to doping controls as members or junior members of the national teams. To facilitate an estimate of the prevalence of doping and illicit drug abuse, the athletes were either issued an anonymous standardized questionnaire (SQ; n = 1394) or were interviewed using randomized response technique (RRT; n = 480). A two-sided z-test was used to compare the SQ and RRT results with the respective official German NADA data on the prevalence of doping. Official doping tests reveal 0.81% (n = 25,437) positive test results, whereas with RRT 6.8% (n = 480) of questioned athletes confessed to having practiced doping. SQ and RRT both revealed a incidence of about 7% for illicit drug use. Therefore, for the first time, data from official doping tests are shown to underestimate the true prevalence of doping in elite sports by greater than a factor of eight. Consequently, implementing RRT before and after anti-doping measures might be a promising method for evaluating the effectiveness of anti-doping programs

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Anal Bioanal Chem 2010 **396** (7) 2479
Characterization of two major urinary metabolites of the PPAR -agonist GW1516 and implementation of the drug in routine doping controls
Starting in January 2009, the World Anti-Doping Agency list of prohibited substances and methods of doping includes new therapeutics such as the peroxisome-proliferator-activated receptor (PPAR)-δ agonist GW1516, which is categorized as a gene doping substance. GW1516 has already completed phase II and IV clinical trials in respect of dyslipidemia and the regulation of the lipoprotein transport in metabolic syndrome conditions. However, its potential to also improve athletic performance has also been recognized and a ban of this compound in elite sport has been imposed. It is believed to enhance expression of genes associated with oxidative metabolism. This has resulted in a modified substrate preference that has moved from carbohydrate to lipid consumption. Recently, two presumably mono-oxygenated and bisoxygenated urinary metabolites of GW1516 have been described. These might be employed as target analytes for doping control purposes after full characterization. In the current work, phase I metabolism was simulated by *in vitro* assays employing human liver microsomal fractions yielding the same oxygenation products. This has been suceeded by chemical synthesis of the assumed structures of the two abundant metabolic reaction products. These facilitated the identification and characterization of mono-oxygenated and bisoxygenated metabolites (sulfoxide and sulfone, respectively) as supported by high-resolution/high-accuracy mass spectrometry with higher-energy collision-induced dissociation, tandem mass spectrometry, and nuclear magnetic resonance spectroscopy. Urine samples are the preferred matrix for doping control purposes. Consequently, a protocol to detect the new target GW1516 in sports drug testing samples has been developed in accordance to conventional screening procedures based on enzymatic hydrolysis and liquid-liquid extraction followed by liquid chromatography, electrospray ionization, and tandem mass spectrometry.

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Anal Bioanal Chem 2010 **396** (8) 2899
Quantification of urinary AICAR concentrations as a matter of doping controls
Enhancing endurance in elite sports is crucial in modern sports science. Recently, a new class of prohibited substances termed gene doping has become apparent to doping control laboratories. Adenosine monophosphate activated protein kinase activator 5-amino-4-imidazolecarboxyamide ribonucleoside (AICAR) has been shown to significantly promote endurance even in sedentary mice after treatment. AICAR is an endogenous compound in healthy humans and thus considerable amounts are present in the circulation and excreted into urine. The present work was commenced in order to provide reference values for renally cleared AICAR in elite athletes. Sample preparation consisting of gentle dilution of native urine. Analysis was by means of isotope-dilution liquid chromatography (analytical column: C6-phenyl) coupled to tandem mass spectrometry. Doping control specimens from 499 athletes were analysed and AICAR concentrations in urine determined. The mean AICAR value for all samples was 2,186 ng/ml with a standard deviation of 1,655 ng/ml. Concentrations were noted to differ in respect of gender, type of sport and type of sample collection (in competition/out of competition). The procedure was fully validated for quantitative purposes considering the parameters linearity, inter- (12%, 7% and 10%) and intraday precision (14%, 9% and 12%) at low, mid and high concentration, robustness, accuracy (approx. 100%), limit of quantification (100 ng/ml), stability and ion suppression effects by employing an in-house synthesised $^{13}C_5$-labelled AICAR as internal standard

3 Steroids

Luosujarvi L, Haapala M, Thevis M, Saarela V, Franssila S, Ketola RA, Kostiainen R, Kotiaho T// Univ Helsinki, Dept Chem, Anal Chem Lab, POB 55, FI-00014 Helsinki, Finland
J Am Soc Mass Spectrom 2010 **21** (2) 310
Analysis of selective androgen receptor modulators by gas chromatography-microchip atmospheric pressure photoionization-mass spectrometry
The analysis of three 2-quinolinone-derived selective androgen receptor modulators (SARMs) has been achieved by the development of a gas chromatography-microchip atmospheric pressure photoionization-mass spectrometric (GC-microAPPI-MS) technique. SARMs were analyzed in spiked urine samples, which were hydrolyzed and derivatized with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide before analysis. Trimethylsilyl derivatives of SARMs produced both radical cations ($M^{+\bullet}$) and protonated molecules ($[M + H]^+$) in photoionization. Better signal-to-noise ratios (S/N) were produced in MS/MS analysis using the $M^{+\bullet}$ ions as precursor ions than using the $[M + H]^+$ ions Therefore, the $M^{+\bullet}$ ions were chosen for the precursor ions in selected reaction monitoring (SRM) analysis. Limits of detection (LODs) with the technique ranged from 0.01 to 1 ng/ml and these correspond with instrumental LODs of 0.2-20 pg. Limits of quantitation ranged from 0.03 to 3 ng/ml. The mass spectrometric response to the analytes was linear (R > or = 0.995) from the LOQ concentration level up to 100 ng/ml concentration, and intra-day repeatabilities were 5%-9%. Further to the GC-microAPPI-MS study, the proof-of-principle of gas chromatography-microchip atmospheric pressure chemical ionization-Orbitrap MS (GC-microAPCI-Orbitrap MS) was illustrated

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Anal Bioanal Chem 2010 **396** (7) 2503
Evaluation of World Anti-Doping Agency criteria for anabolic agent analysis by using comprehensive two-dimensional gas chromatography-mass spectrometry
A validation study of the comprehensive two-dimensional gas chromatography (GC x GC)-time-of-flight mass spectrometry technique performance for the analysis of the key World Anti-Doping Agency (WADA) anabolic agents in doping control is described. Relative abundance ratio, retention time, identification and other method performance criteria have been examined in the GC x GC format to certify that they comply with those set by WADA. A novel approach to doping analysis has been realized by addressing separation improvement. Rather than increasing the method sensitivity, which is accompanied by making the detector increasingly "blind" to the matrix (as represented by selected ion monitoring mode, high-resolution mass spectrometry (MS) and tandem MS), the capabilities of the technique have been enhanced by the provision of a new "separation" dimension whilst retaining full mass spectral scan information. Other than the requirement for the mass spectral domain that a minimum of three diagnostic ions with relative abundance of 5% or higher in the MS spectra, all other WADA criteria are accomplished by GC x GC operation. The requirement for a minimum of three diagnostic ions results from the necessity to add some degree of specificity to the acquired mass spectrometry data. However, with the proposed full MS scan protocol, the high MS resemblance to the reference compounds provides more than the required three diagnostic ions for an unambiguous identification. This is a development of the present criteria of a full-scan MS method

6 CNS Agents

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Anal Bioanal Chem 2010 **396** (7) 2493
Plasma and urine profiles of ^{9}-tetrahydrocannabinol and its metabolites 11-hydroxy- ^{9}-tetrahydrocannabinol and 11-nor-9-carboxy- ^{9}-tetrahydrocannabinol after cannabis smoking by male volunteers to estimate recent consumption by athletes
The World Anti-Doping Agency prohibited cannabis use in 2004 from all sports competitions. However, since the ban, about half of all positive doping cases in Switzerland have been related to cannabis consumption. The target analyte for urinalysis is 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) with a cutoff of 15 ng/ml. However, the persistence of the metabolite of Δ^9-tetrahydrocannabinol (THC) confounds calculations of the time of consumption or the impact on the physical performance. The aim of this research was to study target analytes with shorter urinary excretion times in light cannabis smokers. Male volunteers (n = 12) smoked a cannabis cigarette standardized to 70 mg THC per cigarette. Plasma and urine specimens were taken up to 8 h and 11 days, respectively. Following hydrolysis, solid-phase extraction

and gas chromatography/mass spectrometry wa employed to determine total THC, 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), and THC-COOH. The limits of quantitation were 0.1-1.0 ng/ml. Eight puffs delivered a mean THC dose of 45 mg. Plasma levels of total THC, THC-OH, and THC-COOH were measured in the ranges 0.2-59.1, 0.1-3.9, and 0.4-16.4 ng/ml, respectively. Peak concentrations were noted at 5, 5-20, and 20-180 min. Urine levels were dtermined in the ranges 0.1-1.3, 0.1-14.4, and 0.5-38.2 ng/ml, peaking at 2, 2, and 6-24 h, respectively. Last detectable levels were at 2-8, 6-96, and 48-120 h. In additon to high to very high THC-COOH levels (245 +/- 1,111 ng/ml), THC (3 +/- 8 ng/ml) and THC-OH (51 +/- 246 ng/ml) were found in 65 and 98% of cannabis-positive athlete's urine samples, respectively. In addition to THC-COOH, pharmacologically active THC and THC-OH should be used as target analytes for doping urine analysis. In respect of light cannabis use, this might facilitate the estimation of more recent consumption which might influence performance during competitions. However, it is not possible to discriminate the original intention of cannabis use, i.e., for recreational or sports doping purposes. Furthermore, the study was conducted on males. Pharmacokinetic data of female volunteers are required to interpret cannabis-positive doping cases of female athletes

7 Equine

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Anal Methods 2010 **2** (1) 17

Glycoprotein microarray for the fluorescence detection of antibodies produced as a result of erythropoietin (EPO) abuse

The commercial availability of recombinant human erythropoietin (rHuEPO), has resulted in significant opportunity for athletes, particularly in endurance sports, to illiegally increase their performance by increasing their aerobic capacity through promoted erythrocyte production and thus oxygen transport. rHuEPO abuse has been confirmed in a number of human sports. However, there exists a possibility that rHuEPO may be employed in animal based sports such as thoroughbred horseracing. Recombinant glycoprotein is similar to that produced naturally and typically may only be measured above background levels up to 4 days after application. Therefore, the direct detection of rHuEPO administration is particularly challenging both with urine and blood samples. However, it is known that an immune response results when horses are treated with rHuEPO. The production of a specific antibody after doping with rHuEPO provides a target analyte. This is not only different to endogenous species but one which remains in the animal for considerably longer than the glycoprotein itself therby substantially prolonging the measurement window. A glycoprotein microarray has been developed which exploits the antibody-antigen interaction to provide a method of detecting rHuEPO abuse in animals through the measurement of erythropoietin (EPO) antibodies (anti-HuEPO antibodies). Three commercially available isoforms of rHuEPO (Eprex®, Aranesp® and NeoRecormon®) were arrayed onto the planar surface of a nitrocellulose-coated microarray slide to function as the capture molecule in the assay. The assay was performed by incubation of the microarray with solutions containing the anti-HuEPO antibody and subsequently by incubation with a fluorescently tagged secondary antibody. This sandwich based assay facilitated the fluorescent based detection of anti-HuEPO antibodies using an array-scanner. The EPO glycoprotein microarray was shown to be specific for anti-HuEPO antibodies. To detect anti-HuEPO antibodies in spiked serum samples an optimal dilution of the serum with buffer of 1:4 was determined. Using Eprex®-10,000 IU as the capture molecule, the lowest concentration of anti-HuEPO antibody which was detected using the microarray was 148 pM, indicating that the developed microarray platform might be used as a screen of EPO abuse

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Anal Bioanal Chem 2010 **396** (7) 2513

Doping control analysis of recombinant human erythropoietin, darbepoetin alfa and methoxy polyethylene glycol-epoetin in equine plasma by nano-liquid chromatography-tandem mass spectrometry

Recombinant human erythropoietin (rhEPO), darbepoetin alfa (DPO) and methoxy polyethylene glycol-epoetin β (PEG-EPO) are synthetic analogues of the endogenous hormone erythropoietin (EPO). They act as erythropoiesis-stimulating agents which stimulate the production of red blood cells. They are commercially available for the treatment of anaemia in humans but have been abused with performance-enhancing effects on human athletes due to their stimulation of red blood cell production, thereby improving delivery of oxygen to the muscle tissues. Their performance-enhancing effects on horses has not been proved. However, these substances are believed to be correspondingly performance enhancing and have indeed been applied undeclared to horses. Consequently, these protein-based drugs are forbidden by authorities in both human and equine sports. The International Olympic Committee (IOC) and World Anti Doping Agency (WADA) designated technique for the confirmation of rhEPO and/or DPO (rhEPO/DPO) in human urine is based upon electrophoresis in combination with Western blotting. However, a limitation of the WADA procedure is the absence of definitive mass spectral data for the confirmation of a positive finding. In a recent paper, a liquid chromatography-tandem mass spectrometry (LC/MS/MS) approach for the detection and confirmation of rhEPO/DPO in equine plasma has been reported. However, it has not been successful in accomplishing the necessary sensitivity. Herein, a procedure is described for the detection and confirmation of rhEPO/DPO and also the newly released PEG-EPO, in equine plasma. The method includes immunoaffinity extraction with anti-rhEPO antibody-coated Dynabeads and subsequently trypsin digestion. The injected extract was additionally purified and concentrated using an on-line trap column in the nano-LC system. Detection and confirmation were accomplished by monitoring a unique peptide segment of rhEPO/DPO/PEG-EPO with nano-liquid chromatography-tandem mass spectrometry provided with a nanospray ionisation source working in the selected reaction monitoring mode. rhEPO, DPO and PEG-EPO may be confirmed at 0.1, 0.2 and 1.0 ng/ml, respectively, in equine plasma

8 Recreational Drugs - General

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Anal Bioanal Chem 2010 **396** (7) 2393

Determination of 19 drugs of abuse and metabolites in whole blood by high-performance liquid chromatography-tandem mass spectrometry

Nineteen drugs of abuse and metabolites in whole blood were determined by a high-performance liquid chromatography (LC)-tandem mass spectrometry (MS/MS) approach which has been developed and validated. Compounds included were: amphetamine, methylenedioxyamphetamine, methylenedioxyethylamphetamine, methylenedioxymethamphetamine, methamphetamine, cocaine, benzoylecgonine, morphine, 6-acetylmorphine, codeine, methadone, buprenorphine, norbuprenorphine, ketobemidone, tramadol, *O*-desmethyltramadol, zaleplone, zolpidem, and zopiclone. Sample pretreatment consisted of solid-phase extraction using mixed-mode columns (Isolute Confirm HCX). Deuterated analogues were employed as internal standards for all analytes, except for ketobemidone and *O*-desmethyltramadol. Analytes were separated by a methanol/ammonium formate gradient using high-performance LC (Agilent HPLC 1100) with a 3 mm x 100 mm Varian Pursuit 3 C_{18} column, 3-µm particle size, and were quantified by MS/MS (Waters Quattro micro tandem quadrupole mass spectrometer) using multiple reaction monitoring in positive mode. Two transitions were employed for all analytes with the exception of tramadol and *O*-desmethyltramadol. Run time, including the equilibration time, was 35 min. Responses were linear for all analytes over the range investigated, with $R^2 > 0.99$. One-point calibration was demonstrated to be adequate following validation, thereby saving analysis of multiple calibrators. Limits of quantification (LOQs) for the analytes ranged from 0.0005 to 0.01 mg/kg. Absolute recoveries were from 34 to 97%, except for zaleplone (6%). Both the interday precision and the intraday precision were less than 15% (20% at the LOQ) for all analytes, except buprenorphine, norburprenorphine, and zaleplone (less than 18%). Accuracy (bias) was within +/-15% (+/-20% at the LOQ) for all analytes, except MDMA and *O*-desmethyltramadol (within +/-19%). No ion suppression or enhancement was noted nor was suppression from coeluted analytes observed. Matrix effects were noted to be less than 23% for all analytes, except zopiclone (64%). High-concentration and low-concentration quality control samples produced acceptable values. This approach has been tried in international proficiency test schemes with good results. This LC-MS/MS technique provides a simple, specific, and sensitive solution for the quantification of some of the most frequent drugs of abuse and their metabolites in whole blood. The approach was successfully employed in 412 forensic cases from October 2008 to mid February 2009, where 267 cases were related to zero-tolerance traffic legislation

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Forensic Sci Int 2010 **196** (1-3) 70

Semi-quantitative analysis of drugs of abuse, including tetrahydrocannabinol in hair using aqueous extraction and immunoassay

A semi-quantitative analytical screening approach to determine cocaine, amphetamines, opiates, and Δ^9-tetrahydrocannabinol in hair has been developed. The technique utilizes an aqueous extraction buffer, uses only 10mg of hair, requires 2h of incubation for the extraction to occur, and multiple drug classes may be screened with enzyme linked immunosorbent assays. Hair calibration standards were prepared close to the recommended cut-off concentrations of the Society of Hair Testing. All drug classes demonstrated excellent linearity over the concentration range tested. This indicates that immunochemical screening may be employed in a semi-quantitative mode for hair analysis by utilising an aqueous buffer, rapid extraction and a small amount of hair

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Forensic Sci Int 2010 **196** (1-3) 18

High throughput analysis of drugs of abuse in hair by combining purposely designed sample extraction compatible with immunometric methods used for drug testing in urine

Hair routinely necessitates somewhat complex treatment before drugs are

amenable to analysis by either immunological and/or chromatographic coupled to mass spectrometry methods. Immunological methods employed are usually specific for hair analysis because analytes present in this matrix are not necessarily reflective of urine. Recently, Comedical s.a.s. laboratories started marketing reagents (VMA-T) specifically designed for hair sample treatment but which are suitable for current immunometric methods used for urine drug testing. This is achievable because some analytes (6-MAM and cocaine) present in hair after sample treatment are converted to those detected in urine (morphine and benzoylecgonine). A correlation study for several drug classes has been performed in two laboratories with 32 clinical and 12 spiked drug free (controls) hair samples. It demonstrates that implementation of this approach with clinical chemistry analyzers is simple. It also shows that data obtained by different operators and instruments are comparable and reproducible. The primary benefit of the VMA-T approach is the possibility to simultaneously extract the main drug classes from hair, in a period of time lower than 2h. Furthermore, it is compatible with immunological methods applied in urine drug testing

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Forensic Sci Int 2010 **196** (1-3) 22

Exposure to psychoactive substances in women who request voluntary termination of pregnancy assessed by serum and hair testing

Significant health and socioeconomic impact is derived from the worldwide phenomenon of drug abuse. In addition, it is of special concern in women of reproductive age and, in particular, pregnant women. An investigation has been carried out into the prevalence of drug use by serum and hair testing on a cohort of pregnant women who decided voluntarily to interrupt their pregnancy at 12th week of gestation. In addition, the relationship between drug exposure and induced abortions (IA), repeated IA and contraception was examined. Research was performed in an obstetrics clinic authorised to carry out IA in Murcia, Spain over an 18 months period (2007-2009). Other than serum and/or hair testing, the 142 women enrolled in the study completed a detailed questionnaire in respect of drug, alcohol and tobacco use in the previous 3 months. Serum and hair analyses were performed with gas chromatography-mass spectrometry assays. Overall, hair and serum samples showed a 30% positive response to drugs of abuse. Of these samples, 20.4, 14.1, 4.2 and 1.4% were positive for cannabinoids, cocaine, opiates, and MDMA, respectively and with polydrug use in 5.6% cases. In this cohort, a positive association wasnoted between drug use and repeated IA. The results demonstrate the necessity for promoting pregnancy planning in young women in general, especially when consuming psychoactive substances as well as promoting safe and accessible contraception in women of reproductive age. It is suggested that in women requesting IA, specific drug abuse counselling should be implemented

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Forensic Sci Int 2010 **196** (1-3) 27

Prenatal hair development: Implications for drug exposure determination

Neonatal hair facilitates the determination of *in utero* drug exposure. This paper provides a review the physiological development of prenatal hair follicle and hair production. The significance of the mechanisms and timing of hair development *in utero* is vital to effectively assess the time window of exposure following the toxicological analysis of neonatal hair

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Anal Bioanal Chem 2010 **396** (7) 2461

Analytical evaluation of a rapid on-site oral fluid drug test

A rapid, on-site oral fluid test which may be employed in police controls to detect impaired drivers is necessary. Two oral fluid samples were collected from 250 subjects, one with the Varian Oralab6 and the other with the StatSure Saliva Sampler. The Oralab6 can detect six drug types and on-site results were obtained within 10 to 15 min. Dugs identifiable include amphetamines, methamphetamine, cocaine, opiates, Δ^9-tetrahydrocannabinol (THC), and phencyclidine (PCP). Samples collected with StatSure were analyzed using liquid chromatography-tandem mass spectrometry after liquid-liquid extraction. These data were used as a reference to determine prevalence, sensitivity, and specificity. Two cut-off values were employed in the evaluation. Varian cut-off values were: amphetamine 50 ng/ml, cocaine 20 ng/ml, opiates 40 ng/ml, and THC 50 ng/ml. DRUID cut-offs were: amphetamine 25 ng/ml, cocaine 20 ng/ml, opiates 20 ng/ml, and THC 1 ng/ml. Employing the first cut-offs, prevalence, sensitivity, and specificity were: amphetamine 10%, 76%, 100%; cocaine 23%, 34%, 100%; opiates 38%, 83%, 94%; and THC 18%, 41%, 99%. DRUID cut-off values produced the following results: amphetamine 14%, 56%, 100%; cocaine 28%, 34%, 100%; opiates 49%, 68%, 98%, and THC 45%, 16%, 99%. Generally good specificity is obtained with the Oralab6. For both cut-offs, sensitivity was low for cocaine and THC. Consequently, the Varian Oralab6 test is not sensitive enough to be deployed for roadside police controls

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J Chromatogr A 2010 **1217** (11) 1748

Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography-ion trap-tandem mass spectrometry

The sensitive determination of several drugs of abuse and some of their metabolites in surface and sewage water samples by an alternative procedure is proposed. Analytes are concentrated with a solid-phase extraction (SPE) sorbent, converted into the corresponding trimethylsilyl derivatives and selectively determined by gas chromatography (GC) with tandem mass spectrometry (MS/MS) detection. Factors affecting the performance of extraction, derivatisation and determination steps are examined systematically. Furthermore, the stability of target analytes in sewage water samples is discussed. Under optimized conditions, water samples were adjusted at pH 8.5 and concentrated using a 200mg OASIS HLB SPE cartridge. Analytes were sequentially eluted with ethyl acetate followed by acetone and silylated using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA). Silylation was complete by 60 min at 80 ºC and the mixture was injected directly in the GC-MS/MS system without further purification. Generally, analytes demonstrated poor stability in sewage water samples. However, once they are submitted to the SPE process, cartridges may be stored at -20 ºC for at least 3 months without significant degradation and/or inter-conversion reactions of illicit drugs. The proposed approach produced recoveries over 74% and LODs between 0.8 and 15 ng/l for river and treated wastewater samples. In the case of raw wastewater slightly worse recoveries, between 63 and 137%, and similar LODs resulted. The presence of several illicit drugs in the aquatic environment was confirmed by analysis of a limited number of waste and surface water samples. The highest levels and most frequent analyte was benzoylecgonine, the main metabolite of cocaine

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Forensic Sci Int 2010 **196** (1-3) 38

Gas chromatography-mass spectrometry assay for the simultaneous quantification of drugs of abuse in human placenta at 12th week gestation

By means of gas chromatography-mass spectrometry (GC/MS), an approach has been developed and validated for the quantification of drugs of abuse in human placenta. Concentration ranges included were 5-500 ng/g for amphetamine, methamphetamine, MDMA, methadone, cocaine, benzoylecgonine, cocaethylene, morphine, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, nicotine, and cotinine. Intra-assay and inter-assay imprecisions were less than 15.7% for lower quality control samples as opposed to 14.9% for those of medium and high quality. Recovery range was 36.2-83.7%. Placenta samples were stored at -80 ºC until analysis. Analytes were found to be stable after three freeze-thaw cycles (samples kept at -20 ºC). This accurate and precise technique has the necessary sensitivity and specificity for the analysis of specimens collected from women who voluntarily terminated their pregnancy at 12th week of gestation. The approach was proved to be robust and accurate for the quantification of the principal recreational drugs of abuse at the end of the first trimester. This is the first report to highlight the presence of drugs of abuse during the first trimester of gestation

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Forensic Sci Int 2010 **196** (1-3) 43

Rapid and simple determination of psychotropic phenylalkylamine derivatives in human hair by gas chromatography-mass spectrometry using micro-pulverized extraction

Five psychotropic phenylalkylamine derivatives (amphetamine, AP; methamphetamine, MA; 3,4-methylenedioxyamphetamine, MDA; 3,4-methylenedioxymethamphetamine, MDMA; norketamine, NKT) in human hair have been investigated by the development and validation of a gas chromatography-mass spectrometric (GC-MS) procedure. Hair samples (10mg) were washed with distilled water and acetone, mechanically pulverized for 1.5 min with a bead mill, and then incubated in 1 ml of methanol under ultrasonication at 50 ºC for 1h. Resulting solutions were evaporated to dryness, derivatized using heptafluorobutyric anhydride (HFBA) at 50 ºC for 30 min before analysis. The linear ranges were 0.1-20.0 ng/mg for AP and MA and 0.05-20.0 ng/mg for MDA, MDMA, and NKT, with the coefficients of determination ($r^2 > 0.9982$). Intra-day and inter-day precisions were within 11.5% and 12.8%, respectively. The intra-day and inter-day accuracies were -4.1% to 5.8% and -6.6% to 4.2%, respectively. The limits of detections (LODs) for each compound were lower than 0.028 ng/mg. Recoveries ranged 78.9-101.2%. Consequently, the approach proved to be effective for the rapid and simple determination of phenylalkylamine derivatives in hair specimens

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Forensic Sci Int 2010 **196** (1-3) 55

Hair analysis for drugs in driver's license regranting. A Swedish pilot study

In Sweden, a person's driving license may be suspended following conviction for a petty drug offence or driving under the influence of drugs. In order to regain their license, the person must demonstrate that he or she has been drug free during a controlled period. This is monitored by analysis of urine samples taken at several occasions. However, the risk of manipulation and of false negative urine samples are not insignificant. Furthermore, many people find it difficult or embarrassing to urinate when observed. Therefore, hair sampling

might be an agreeable alternative to this procedure considering its easy sampling and minimal risk of manipulation. The longer detection window provided by hair may also provide better information to the physician. This research was undertaken to investigate whether clients preferred hair to urine samples. It was also employed to investigate practical and interpretive advantages or disadvantages with hair samples. Ninety-nine hair samples and 198 urine samples were collected from 84 clients during the 12 month study period. Hair samples were divided into either one segment (0-3 cm) or two segments (0-3 and 3-6 cm) dependent upon the length. Hair samples were screened with LC-MS-MS for 20 drugs and positive results confirmed with GC-MS or LC-MS-MS. Results were compared with urine samples obtained on two occasions during the observation period. To cover the period of the urine samples, hair was collected 2 weeks after the second sample. Urine samples were analysed with immunochemical screening. Positive results were confirmed with GC-MS or LC-MS-MS. Analysis of specimens from 74 individuals resulted in negative results for both urine and hair. Hair analysis identified illegal drugs on seven different occasions whereas urine failed to identify any illegal drugs. However, the thresholds employed may still be too high to find intermittent use because clients that admitted to use drugs sporadically presented with drug concentrations lower than the agreed thresholds but greater than the limit of detection. This suggests that the physician should have an understanding and knowledge of the limitations of the screening methods employed. Clients approved of hair sampling considering it a preferable means to demonstrate their drug abstinence than urine. Both clients and the clinicians believed that hair rather than urine sampling was easier. Hair rather than analysis offers several advantages for clinicians assessing suitability for driving license regranting.

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Forensic Sci Int 2010 **197** (1-3) 105
A probability-based sampling approach for the analysis of drug seizures composed of multiple containers of either cocaine, heroin, or cannabis
A probability-based analytical sampling approach to answer questions regarding both content weight and identity of seized containers of cocaine, cannabis, or heroin is described. It employs the Student's t distribution, and, because of the lack of normality in studied populations, the power of the Central Limit Theorem with samples of size 20 to calculate the mean net weights of multiple item drug seizures. Populations examined ranged from 50 to 1200 units. Identity determination employs chemical testing and sampling using the hypergeometric distribution fit to a program macro (created by the European Network of Forensic Science Institutes (ENFSI) Drugs Working Group). Formal random item selection is invoked through use of an Excel-generated list of random numbers. Due to their impact on actual practice, discussions of admissibility, sufficiency of proof, method validation, and harmony with the guidelines of international standardizing bodies are included

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Forensic Sci Int 2010 **197** (1-3) 80
Small volume liquid extraction of amphetamines in saliva
A protocol for analysis of amphetamines, including amphetamine (AM); methamphetamine (MA); 3,4-methylenedioxyamphetamine (MDA); 3,4-methylenemethamphtamine (MDMA) with small volume liquid extraction of saliva is described. Extraction efficiencies were compared between the conventional volume liquid phase extraction (LPE) and the small volume one, in which < 100 µl solvent rather than several milliliters in LPE. Types and volumes of organic solvent employed in the extraction and concentrations of target analytes in aqueous samples were investigated. Data demonstrate that small volume liquid extraction had an enrichment effect on the analytes. Following extraction, the organic phase was either directly drawn out for GC analysis, or partially transferred to another vial for derivatization. Detection limits were less than 5 ng/ml in saliva using GC/MS-SIM after derivatization. RSD (of peak area ratios) was less than 15% for all drug concentrations. The approach was employed in the analyses of saliva collected from amphetamine abusers. It was proven suitable for detecting trace amounts of amphetamines in saliva

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Forensic Sci Int 2010 **196** (1-3) 85
Simultaneous screening and quantification of 52 common pharmaceuticals and drugs of abuse in hair using UPLC-TOF-MS
The simultaneous screening and quantification of 52 drugs in hair was achieved through the development and validation of an UPLC-TOF-MS technique. The most common classes of pharmaceuticals and drugs of abuse such as amphetamines, analgesics, antidepressants, antipsychotics, benzodiazepines, cocaine, ketamine and opioids were analysed. Hair samples were extracted with methanol:acetonitrile:ammonium formate (2 mM, 8% acetonitrile, pH 5.3) overnight at 37 ºC. A Waters ACQUITY UPLC coupled to a Waters Micromass LCT Premier XE time-of-flight mass spectrometer was employed to separate and quantify target drugs. Total chromatographic run time was 17min. Data were treated with the MassLynx software ChromaLynx XS and QuanLynx for automated identification and quantification, respectively. Limits of detection ranged from 0.01 to 0.10 ng/mg using a 10 mg hair sample and the limit of quantification was 0.05 ng/mg for 87% of the analytes. Good linear behaviour was accomplished for most analytes in the range from LOQ to 10 or 25 ng/mg except for the amphetamines. The approach demonstrated acceptable precision and trueness because the obtained CV and BIAS values were < or = 25% for 81% of the analytes. Extraction recoveries for 92% of the analytes were between 84 and 106%. Extraction recoveries for all analytes were better than 60%. The procedure was employed with 15 autopsy hair samples from forensic investigations. It illustrated a wide abuse pattern for many pharmaceuticals and drugs of abuse within a period of less than three months. Data show that the combination of accurate mass and retention time can provide good selectivity, which indicates that the TOF instrument is sufficient for both screening and quantification purposes. In addition, it was noted that screening with the ChromaLynx XS is less sensitive and selective for some analytes than the QuanLynx, particularly at low concentrations

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Anal Bioanal Chem 2010 **396** (7) 2379
Use of liquid chromatography coupled to low- and high-resolution linear ion trap mass spectrometry for studying the metabolism of paynantheine, an alkaloid of the herbal drug Kratom in rat and human urine
Mitragyna speciosa (Kratom in Thai), a Thai medicinal plant is misused as a herbal drug of abuse. The main Kratom alkaloid is mitragynine (MG). Several dehydro analogs could be detected in urine of Kratom users but these were not found in rat urine after administration of pure MG. Whether these compounds were formed from MG only by humans or whether they were metabolites produced from the second abundant Kratom alkaloid paynantheine (PAY), the dehydro analog of MG, was investigated. Therefore, research was undertaken to identify the phase I and II metabolites of PAY in rat urine after administration of the pure alkaloid isolated from Kratom leaves. Liquid chromatography-linear ion trap mass spectrometry provided detailed structure information of the metabolites in the MS^n mode with particularly high resolution. In addition to PAY, the following phase I metabolites could be identified: 9-*O*-demethyl PAY, 16-carboxy PAY, 9-*O*-demethyl-16-carboxy PAY, 17-*O*-demethyl PAY, 17-*O*-demethyl-16,17-dihydro PAY, 9,17-*O*-bisdemethyl PAY, 9,17-*O*-bisdemethyl-16,17-dihydro PAY, 17-carboxy-16,17-dihydro PAY, and 9-*O*-demethyl-17-carboxy-16,17-dihydro PAY. These metabolites demonstrated that PAY was metabolized *via* the same pathways as MG and several were excreted as glucuronides or sulfates. Metabolism studies in rats confirmed that PAY and its metabolites corresponded with the MG-related dehydro compounds detected in urine of the Kratom users. Therefore, PAY and its metabolites may be further markers for a Kratom abuse in addition of MG and its metabolites

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Anal Bioanal Chem 2010 **397** (2) 501
Certification of drugs of abuse in a human serum standard reference material: SRM 1959
A novel standard reference material (SRM) for drugs of abuse in human serum has been produced (SRM 1959). SRM 1959 has been developed as a control material for laboratories carrying out analyses of drugs of abuse in blood in order to assess the accuracy of their techniques. SRM 1959 is a frozen human serum material spiked with seven typical drugs of abuse, namely benzoylecgonine (BZE), methadone (METH), methamphetamine (MAMP), morphine (MOR), nordiazepam (NOR), phencyclidine (PCP), and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-9-COOH). Two independent techniques involving isotope dilution (ID)-gas chromatography/mass spectrometry (GC/MS) and ID-liquid chromatography/mass spectrometry (LC/MS) were employed to quntify the analytes. In the case of THC-9-COOH, an additional measurement using LC/tandem mass spectrometry (LC/MS/MS) was also included. All approaches employed internal standards of isotopically labeled compounds and solid-phase extractions to isolate the analytes from the serum. The GC/MS methods employed different clean-up procedures compared with those used for the LC/MS-based methods. Repeatability with within-set coefficients of variation (CVs) ranged from 0.5% to 4.3% for the GC/MS techniques and from 0.2% to 1.2% for the LC/MS-based protocols. Intermediate precision with between-set CVs for all the procedures ranged from 0.1% to 1.1%. Agreement between the GC/MS and LC/MS approaches ranged from 0.8% to 8.8%. Data from the different procedures were combined to produce the certified concentrations and their expanded uncertainties

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J Clin Pathol 2010 **63** (3) 259
***In utero* drugs of abuse exposure testing for newborn twins**
A case of *in utero* drugs of abuse exposure was investigated and produced discordant results. Inconsistency was noted between urine and meconium and between twin meconium samples. The difference between urine and meconium might be as a result of the differences in detection window, threshold concentration and screening technology. Whereas the disagreement between dizygotic twin meconium samples might be explained by the differences in drug diffusion and placental and fetal biotransformation of drugs. The meconium sample of one twin which had screened negative for benzodiazepines was produced positive results in a confirmation assay with higher sensitivity and a lower

cut-off concentration. Negative screening results of drugs of abuse should be interpreted with caution, taking into account matrix type, reactivity of drugs in the assay and cut-off concentration. Should screening results contradict each other or the clinical scenario, confirmation testing employing more sensitive and specific approaches and lower cut-offs are required

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Anal Bioanal Chem 2010 **396** (7) 2403
LC-MS/MS screening method for designer amphetamines, tryptamines and piperazines in serum
Since the turn of the last century, derivatives of well-known designer drugs plus new psychoactive compounds have been sold on the illicit drug market and have resulted in intoxications and fatalities. A screening approach covering 31 new designer drugs as well as cathinone, methcathinone, phencyclidine, and ketamine (included to complete the screening spectrum) by LC-MS/MS has been developed. Other than phencyclidine and ketamine, all are modified molecular structures of amphetamine, tryptamine, or piperazine. Amphetamine derivatives include cathinone, methcathinone, 3,4-DMA, 2,5-DMA, DOB, DOET, DOM, ethylamphetamine, MDDMA, 4-MTA, PMA, PMMA, 3,4,5-TMA, TMA-6 and members of the 2C group: 2C-B, 2C-D, 2C-H, 2C-I, 2C-P, 2C-T-2, 2C-T-4, and 2C-T-7. AMT, DPT, DiPT, MiPT, DMT, and 5MeO-DMT are within the tryptamine group, BZP, MDBP, TFMPP, mCPP, and MeOPP in the piperazine group. It is possible to identify all 35 substances with an Applied Biosystems LC-MS/MS API 365 TurboIonSpray. Following addition of internal standards and mixed-mode solid-phase extraction the compounds are separated with a Synergi Polar RP column and gradient elution with 1 mM ammonium formate and methanol/0.1% formic acid as mobile phases A and B. Data acquisition was performed in MRM mode with positive electro spray ionization. The assay is selective for all tested substances. Limits of detection were determined by analyzing S/N-ratios and are between 1.0 and 5.0 ng/ml. Matrix effects lie between 65% and 118%, extraction efficiencies range from 72% to 90%

9 Stimulants

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Talanta 2010 **81** (1-2) 455
Recoveries of trace pseudoephedrine and methamphetamine residues from impermeable household surfaces: Implications for sampling methods used during remediation of clandestine methamphetamine laboratories
Contaminants present during and after decontamination of clandestine methamphetamine laboratories pose a risk that requires assessment. It necessitates a connection between the levels of contaminants measured and those actually present at the scene. GC-MS of derivatized samples were employed to determine recoveries of pseudoephedrine and methamphetamine from glass, stainless steel, and a range of impermeable surfaces likely to be found in a clandestine laboratory. On surfaces which had been cleaned prior to drug deposition, wiping with water-dampened filter paper recovers 60-80% of pseudoephedrine immediately after deposition. At least 50% of the pseudoephedrine is recovered when still present on a surface after 2 days and deposited at a surface concentration of 2.5 μg/100 cm^2. Wiping with methanol-dampened filter paper could recover 60-90% of the methamphetamine immediately after deposition. Also it could recover at least 50-60% of the methamphetamine still present after 2 days when 0.6 μg/100 cm^2 was initially deposited on the surface. Recoveries were lower from surfaces that had not been pre-cleaned. Methamphetamine and pseudoephedrine exhibited notable volatility in both the free base and hydrochloride forms in an enclosed format showing up to half the recovered drug being present on a glass plate held about 4mm above a substrate contaminated with one of the drugs at the above surface concentrations after 2 days. Therefore, it is necessary to remove any visible bulk contaminants and obvious pseudoephedrine or methamphetamine-contaminated surfaces prior to heating, ventilation or sealing of a clandestine laboratory to avoid redistribution of material around the site. A revised method for pseudoephedrine analysis has been developed that could also detect the pseudoephedrine-formaldehyde adduct which might form from trace pseudoephedrine present at clandestine laboratories

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Forensic Sci Int 2010 **197** (1-3) 59
Chemical analysis of four capsules containing the controlled substance analogues 4-methylmethcathinone, 2-fluoromethamphetamine, -phthalimidopropiophenone and *N*-ethylcathinone
In August 2007, four capsules were anonymously delivered to the Royal Adelaide Hospital, South Australia. They contained white powders and supposedly originated from an Israel-based Internet company "Neorganics". Capsules were analysed and the active components were identified. These included 4-methylmethcathinone, 2-fluoromethamphetamine, α-phthalimidopropiophenone and *N*-ethylcathinone. All of these substances were unlisted within South Australian controlled substance regulations. Both GCMS and NMR data for 4-methylmethcathinone and α-phthalimidopropiophenone are presnted. These have previously received little attention. Also presented are the vapour- and condensed-phase infrared spectra (IR) of 4-methylmethcathinone which have not been reported in the literature previously. The issues surrounding whether these chemicals might be classified as controlled substance analogues is discussed. In addition, the likely impact this could have on prosecutions of individuals distributing these products

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J Chromatogr Sci 2010 **48** (3) 224
Investigation of pencil leads fiber efficiency for SPME of trace amount of methamphetamine from human saliva prior to GC-MS analysis
The effectiveness of head-space solid-phase microextraction (HS-SPME) of methamphetamine (MAMP) with pencil lead fiber from aqueous standard solutions without chemical derivatization prior to gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses was examined. Experimental parameters, for example, extraction temperature and time, sample pH, and salting out were studied and optimized. Under optimum conditions, the efficiency of this fiber was compared with polyacrylate (PA) commercial fiber, which is most selective for volatile and semi-volatile compounds. Data illustrate the suitability of modified pencil-lead fiber for sampling of the studied compound from aqueous solutions. Under optimum conditions, the calibration plot was linear in the range of 40-8000 ng/ml (r = 0.998), and the limit of detection was 27 ng/ml (n = 3). The proposed method was successfully employed for HS-SPME of MAMP analysis of 200 μl human saliva which has been spiked with trace amounts of MAMP (160 ng/ml) followed by GC-MS monitoring

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J Am Soc Mass Spectrom 2010 **21** (2) 290
Fast quantitative detection of cocaine in beverages using nanoextractive electrospray ionization tandem mass spectrometry
Effervescent beverage fluids were manually sprayed into the primary ion plume created by using a nanoelectrospray ionization source for direct ionization without any sample pretreatment. Analyte ions of interest were guided into an ion trap mass spectrometer for tandem mass analysis. Normal functional ingredients (e.g., vitamins, taurine, and caffeine, etc.) and spiked impurity (e.g., cocaine) in various beverages, such as Red Bull energy drink, Coco-cola, and Pepsi samples were rapidly identified within 1.5 s. Limit of detection was noted to be 7-15 fg (S/N = 3) for cocaine in different samples using the characteristic fragment (m/z 150) observed in the MS^3 experiments. Typical relative standard deviation and recovery of this approach were 6.9%-8.6% and 104%-108% for direct analysis of three actual samples. This demonstrates that nanoextractive electrospray ionization tandem mass spectrometry is a useful approach for fast screening of cocaine in beverages

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Forensic Sci Int 2010 **196** (1-3) 59
Pharmacokinetics of methylphenidate in oral fluid and sweat of a pediatric subject
The treatment of attention-deficit hyperactivity disorder (ADHD) in children and adolescents is frequently with methylphenidate (MPH), a stimulant medication. MPH measurement iis necessary to monitor consumption and misuse in situations where detection of recent abuse is of interest. Therapeutic monitoring for this drug is essentially lacking. Alternative biological matrices, such as oral fluid and sweat, might be analyzed for noninvasive assessment of short- and long-term history of drug use. The excretion profile in oral fluid and sweat from a 12-year-old boy treated with the extended release drug formulation of MHP has been investigated in addition to metabolite, ritalinic acid (RA). Concentrations of MPH and RA in oral fluid, sweat and plasma were measured by liquid chromatography-mass spectrometry. Oral fluid-to-plasma ratio at each time interval was calculated at the start of the treatment and correlated with salivary pH. Excretion of MPH in sweat patches, collected up to 24h with PharmChek patches was also determined. MPH and RA were both detected in oral fluid with a pharmacokinetic profile similar to that in plasma. Oral fluid peak concentrations of MPH ranged between 13.5 and 30.9 ng/ml at 3.0 h after drug intake. Oral fluid-to-plasma MPH ratio between 13.1 and 3.2 revealed an accumulation of the drug in oral fluid. Conversely, RA was found in oral fluid at peak concentration (23.4-62.9 ng/ml) equivalent to one-tenth of those found in plasma. Concentration profiles of MPH and RA in oral fluid were quite constant during the four weeks of drug administration. In sweat, MPH was detected for the first time at 5h after drug administration (range: 9.3-11.2 ng/patch) up to 24h (range: 29.8-38.7 ng/patch). RA was not detected in the sweat patches during the 24h time of collection. Data indicate that measurement of MPH in oral fluid may be used as a potential alternative to drug monitoring in plasma. Furthermore, where detection of recent abuse is required, MPH may be measured in sweat patches as a non-invasive approach to monitoring consumption

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Anal Chim Acta 2010 **661** (1) 42

Monolithic silica spin column extraction and simultaneous derivatization of amphetamines and 3,4-methylenedioxyamphetamines in human urine for gas chromatographic-mass spectrometric detection
The simultaneous extraction and derivatization of amphetamines (APs) and 3,4-methylenedioxyamphetamines (MDAs) in human urine has been achieved throught the development of a simple, sensitive, and specific procedure employing gas chromatography-mass spectrometry with a monolithic silica spin column. All procedures, for example, sample loading, washing, and elution were performed by centrifugation. APs and MDAs in urine were adsorbed on the monolithic silica and derivatized with propyl chloroformate in the column. Methamphetamine-d_5 was used as an internal standard. Linear ranges were 0.01-5.0 μg/ml for methamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA) and 0.02-5.0 μg/ml for amphetamine (AP) and 3,4-methylenedioxyamphetamine (MDA) (coefficient of correlation > or = 0.995). The recovery of APs and MDAs in urine was 84-94%, and the relative standard deviation of the intra- and interday reproducibility for urine samples containing 0.1, 1.0, and 4.0 μg/ml of APs and MDAs ranged from 1.4% to 13.6%. The lowest detection limit (signal-to-noise ratio > or = 3) in urine was 5 ng/ml for MA and MDMA and 10 ng/m for AP and MDA. The described protocol may be employed to carry out simultaneous extraction and derivatization on spin columns which have been loaded with a small quantity of solvent by using centrifugation

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Anal Bioanal Chem 2010 **396** (5) 1703
Hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) determination of cocaine and its metabolites benzoylecgonine, ecgonine methyl ester, and cocaethylene in hair samples
The analysis of cocaine and its metabolites benzoylecgonine (BE), ecgonine methyl ester (EME), and cocaethylene (CE) in hair samples has been accomplished through the development and validation of a hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) technique. Firstly, decontamination was performed. The aliquot of hair was briefly rinsed with 2 ml dichloromethane, then was washed three times with 10 ml 0.01 M phosphate buffer, pH 6, for 15 min, followed by 2 ml 2-propanol for less than 2 min, and, finally, a last rinse with 2 ml dichloromethane was again done. Cocaine compounds were extracted from 10 mg of hair by incubation with 2 ml 0.1 M HCl at 50 ºC for 12 h and purified by solid phase extraction with Oasis MCX cartridges. Analysis was carried out by LC-MS/MS using an Atlantis HILIC silica chromatographic column. The procedure was fully validated. Linearity was confirmed over the concentration range 0.020-10.0 ng/mg for cocaine (COC), 0.010-10.0 ng/mg for BE and CE, and 0.005-2.0 ng/mg for EME, and the correlation coefficients were all > 0.99. Extraction efficiency was > 70% for all analytes. Limits of detection were 0.0005 ng/mg for CE and 0.001 ng/mg for the other analytes (COC, BE, and EME). Lower limits of quantification were the lowest points of the calibration curves with acceptable accuracy and precision (coefficient of variation < / = 20%). Intra- and inter-day imprecision ranged between 1.5% and 9.5% and 0.7% and 12.6%, respectively. Intra- and inter-day inaccuracy ranged from 0.5% to 12.3% and from 0.7% to 7.1%, respectively. In respect of matrix effects, suppression was < 27.5% in all cases. The protocol was employed in the analysis of several samples derived from forensic cases

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J Am Soc Mass Spectrom 2010 **21** (4) 564
Quantitative MALDI-MS^n analysis of cocaine in the autopsied brain of a human cocaine user employing a wide isolation window and internal standards
The detection of drugs frquently necessitates extensive sample preparation. Tissue is first homogenized, followed by drug extraction, before the extracts are finally analyzed by LC/MS. Directly analysis drugs in intact tissue would preclude any difficulties introduced by sample pretreatment. The quantification of cocaine present in postmortem brain tissue of a chronic human cocaine user has been accomplished by the development of a matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS^n) procedure. It is demonstrated that tandem mass spectrometry (MS^2 and MS^3) increases selectivity, which is essential for differentiating analyte ions from those of the background, for example, matrix clusters and endogenous compounds found in brain tissue. In addition, it is shown that the use of internal standards corrects for signal variability during quantitative MALDI. These may result from inhomogeneous crystal formation, inconsistent sample preparation, and laser shot-to-shot variability. Development of the MALDI-MS^n approach facilitates a single MS^3 experiment that employs a wide isolation window to separate both analyte and internal standard target ions. This approach is demonstrated result in improved precision [approximately 10-20 times reduction in percent relative standard deviation (%RSD)] for quantitative analysis compared with the use of two alternating MS^3 experiments that separately isolate the target analyte and internal standard ions

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Anal Bioanal Chem 2010 **396** (7) 2435
Validation of a fast screening method for the detection of cocaine in hair by MALDI-MS
A novel method of screening for cocaine in hair, based on matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry (MS), have been evaluated in respect of sensitivity and specificity. The procedure involves a rapid extraction procedure which employs the shaking 2.5 mg pulverised hair at high frequency in the presence of an acidic solution (160 μl of water, 20 μl of acetonitrile and 20 μl of 1 M trifluoroacetic acid) and a stainless-steel bullet. After centrifugation, the supernatant is dried under a nitrogen stream, and the residue is reconstituted in 10 μl of methanol/trifluoroacetic acid (7:3; v/v). One microlitre of the extract is positioned on a MALDI sample holder previously scrubbed with graphite. An α-cyano-4-hydroxycinnamic acid (matrix) solution is electrosprayed over the dried sample surface to produce a uniform distribution of matrix crystals. The identification of cocaine is achieved by post-source decay experiments performed on its MH^+ ion (*m/z* 304), with a limit of detection of 0.1 ng/mg of cocaine. Hair samples (n = 304) were analysed in parallel by MALDI-MS and a reference gas chromatography-MS method. Data produced show specificity and sensitivity of 100% for MALDI-MS. Cocaine was easily detectable even when hair samples contained particularly low cocaine levels (< 0.5 ng/mg)

10 Hallucinogens

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Forensic Sci Int 2010 **196** (1-3) 10
Hair analysis for ^{9}-tetrahydrocannabinolic acid A—new insights into the mechanism of drug incorporation of cannabinoids into hair
In hair analysis, the ability to distinguish between external contamination and incorporation of drugs or their metabolites from inside the body *via* blood, sweat or sebum is a priority when interpreting analytical results. In hair analysis for cannabinoids, the most common target is Δ^9-tetrahydrocannabinol (THC). In addition, cannabidiol (CBD) and cannabinol (CBN) may be determined. Following repeated external contamination by cannabis smoke, these analytes may to be found in hair even after performing multiple washing steps. A widely accepted approach to verify active cannabis consumption is the analysis of hair extracts for the oxidative metabolite 11-nor-9-carboxy-THC (THC-COOH). The acidic nature of this metabolite indicates a lower rate of incorporation into the hair matrix compared with THC. However, to date, it is not fully understood why hair concentrations of THC-COOH are generally found to be much lower (mostly < 10 pg/mg) than the corresponding THC concentrations. Δ^9-tetrahydrocannabinolic acid A (THCA A) is the preliminary end product of the THC biosynthesis in the cannabis plant. Unlike THC, it is non-psychoactive and may be regarded as a 'precursor' of THC. It is largely decarboxylated when heated or smoked. Herein, for the first time, it is shown THCA A is not only detectable in blood and urine of cannabis consumers but also in THC positive hair samples. In a preliminary study, it appeared that after oral intake of THCA A on a regular basis, no significant incorporation into hair occurred. Therefore, it is apparent that THCA A in hair is as a result external contamination e.g. by side stream smoke. Elevated temperatures during the analytical procedure, particularly under alkaline conditions, may result in decarboxylation of THCA A and accordingly increase THC concentrations in hair. Furthermore, it should be considered that in hair samples tested positive for THCA A at least a part of the 'non-artefact' THC probably also derives from external contamination because in condensate of cannabis smoke both THC and THCA A are present in relevant amounts. Side stream smoke contamination might explain the great differences in THC and THC-COOH hair concentrations generally found in cannabis users

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J Chromatogr B 2010 **878** (9-10) 815
Gas chromatography-ion trap mass spectrometry method for the simultaneous measurement of MDMA (Ecstasy) and its metabolites, MDA, HMA, and HMMA in plasma and urine
An optimized gas chromatography-ion trap mass spectrometry (GC-IT/MS) procedure with electron impact ionizationhas been employed in the investigation of 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) abuse and its main metabolites in body fluids. These analytes necessitate very robust methods with high sensitivity and wide linearity ranges in order to facilitate quantification. Sample preparation involved enzymatic hydrolysis of urine and plasma to cleave conjugates, SPE extraction, and a derivatization step. The approach was fully validated with rat plasma and urine. Over a wide concentration range, linearity was accomplished for MDMA, and the metabolites 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA). Limits of quantification were 2 ng/ml in plasma and 3.5 ng/ml in urine using a selected ion monitoring detection mode. Selectivity, accuracy, precision, and recovery achieved the required parameters for method validation. The simultaneous

quantification of MDMA, MDA, HMA and HMMA in the studied matrices may be achieved with this GC-IT/MS approach which provides high sensitivity and adequate performance characteristics

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Anal Chem 2010 **82** (7) 2826

Dynamic planar solid phase microextraction-ion mobility spectrometry for rapid field air sampling and analysis of illicit drugs and explosives

The inlet of an ion mobility spectrometer (IMS) has been modified with a preconcentration device that targets the volatile chemical signatures associated with illicit drugs and explosives (high and low). Reported for the first time is a fast and sensitive approach for dynamic sampling of large volumes of air using planar solid phase microextraction (PSPME) incorporating a high surface area for absorption of analytes onto a sol-gel polydimethylsiloxane (PDMS) coating for direct thermal desorption into an IMS. This device facilitates high extraction efficiencies because of strong retention properties at ambient temperature. Therefore, the detection of analyte concentrations in the parts per trillion range is possible when as low as 3.5 litres of air are sampled over the course of 10 s (absolute mass detection of less than a nanogram). The appartus was employed to sample the headspace over a number of compounds. 3,4-Methylenedioxymethamphetamine (MDMA) tablets resulted in the detection of 12-40 ng of piperonal. High explosives (Pentolite) revealed 0.6 ng of 2,4,6-trinitrotoluene (TNT), and from low explosives (several smokeless powders) 26-35 ng of 2,4-dinitrotoluene (2,4-DNT) and 11-74 ng of diphenylamine (DPA) were detected

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Anal Bioanal Chem 2010 **397** (2) 603

Validation of a two-dimensional gas chromatography mass spectrometry method for the simultaneous quantification of cannabidiol, ^{9}-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC in plasma

The monitoring of cannabinoid pharmacotherapy and illicit cannabis use has been achieved with a sensitive analytical technique. Compounds were extracted from 1 ml plasma by solid-phase extraction, derivatized with *N,O*-bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane, and analyzed by two-dimensional gas chromatography mass spectrometry (2D-GCMS) with cryofocusing. The simultaneous quantification of sub-nanogram concentrations of cannabidiol (CBD), Δ^{9}-tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) in plasma was accomplished. The lower calibration curve was linear from 0.25-25 ng/ml for CBD and THC, 0.125-25 ng/ml for 11-OH-THC and 0.25-50 ng/ml for THCCOOH. A second higher linear range from 5-100 ng/ml, achieved through modification of injection parameters, was validated for THC, 11-OH-THC, and THCCOOH. It was only implemented if concentrations exceeded the lower curve upper limit of linearity. This approach avoided laborious re-extraction by allowing the same sample to be re-injected for quantification on the high calibration curve. Intra- and inter-assay imprecision, determined at four quality control concentrations, were < or = 7.8% CV. Analytical bias was within +/-9.2% of target and extraction efficiencies were > or = 72.9% for all analytes. Compounds were stable when stored at 22 ^{o}C for 16 h, 4 ^{o}C for 48 h, after three freeze-thaw cycles at -20 ^{o}C and when stored on the autosampler for 48 h. This sensitive and specific 2D-GCMS method provides a new approach simultaneously quantifying CBD, THC and metabolite biomarkers. It is suitable for use in clinical medicine, forensic toxicology, workplace drug testing, and driving under the influence of drugs programs

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Drug Alcohol Depend 2010 **106** (1) 65

^{9}-Tetrahydrocannabivarin testing may not have the sensitivity to detect marijuana use among individuals ingesting dronabinol

Δ^{9}-Tetrahydrocannabivarin (THCV) is a plant cannabinoid. A study was undertaken to determine whether it is a suitable analyte to detect recent marijuana use in cannabis dependent patients. Previously, it has been suggested that smoking illicit cannabis will result in a positive THCV urinalysis. However, oral ingestion of therapeutic THC such as dronabinol will result in a negative THCV urinalysis. This will facilitate discrimination between pharmaceutical THC and illicit marijuana consumption. A double-blind placebo-controlled trial was undertaken to determine the efficacy of dronabinol in cannabis dependence. All 117 patients produced a positive urine for the marijuana metabolite 11-nor-Δ^{9}-THC-9-carboxylic acid; THC-COOH. However, 50% had an undetectable (< 1 ng/ml) THCV-COOH test. This indicates that THCV may not be a sensitive enough analyte to detect recent marijuana use in all heavy marijuana users. Moreover, its absence may not discriminate between illicit marijuana use and oral ingestion of THC products such as dronabinol. It is suggested that the lack of THCV detection might be due to the variability of available cannabis strains smoked by marijuana users in community settings

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J Chromatogr A 2010 **1217** (9) 1513

Simultaneous quantification of cannabinoids and metabolites in oral fluid by two-dimensional gas chromatography mass spectrometry

The simultaneous identification and quantification of Δ^{9}-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), and metabolites 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) in oral fluid has been accomplished throught the development and validation of a two-dimensional gas chromatography mass spectrometry procedure. Simultaneous analysis was difficult because of different physicochemical characteristics and concentration ranges. Neutral analytes, such as THC and CBD, were present in ng/ml, rather than pg/ml concentrations, as observed for the acidic THCCOOH biomarker in oral fluid. THCCOOH was not present in cannabis smoke, clearly separating cannabis use from passive smoke exposure. THC, 11-OH-THC, THCCOOH, CBD, and CBN quantification was accomplished in a single oral fluid specimen collected with the Quantisal device. One ml oral fluid/buffer solution (0.25 ml oral fluid and 0.75 ml buffer) was introduced to conditioned CEREX Polycrom THC solid-phase extraction (SPE) columns. After washing, THC, 11-OH-THC, CBD, and CBN were eluted with hexane/acetone/ethyl acetate (60:30:20, v/v/v), derivatized with *N,O*-bis-(trimethylsilyl)trifluoroacetamide and quantified by two-dimensional gas chromatography electron ionization mass spectrometry (2D-GCMS) with cold trapping. Acidic THCCOOH was separately eluted with hexane/ethyl acetate/acetic acid (75:25:2.5, v/v/v), derivatized with trifluoroacetic anhydride and hexafluoroisopropanol, and quantified by the more sensitive 2D-GCMS-electron capture negative chemical ionization (NCI-MS). Linearity was 0.5-50 ng/ml for THC, 11-OH-THC, CBD and 1-50 ng/ml for CBN. The linear dynamic range for THCCOOH was 7.5-500 pg/ml. Intra- and inter-assay imprecision as percent RSD at three concentrations across the linear dynamic range were 0.3-6.6%. Analytical recovery was within 13.8% of target. This novel SPE 2D-GCMS procedure achieved efficient quantification of five cannabinoids in oral fluid, including pg/ml concentrations of THCCOOH by combining differential elution, 2D-GCMS with electron ionization and negative chemical ionization. This protocol is to be employed in the quantification of cannabinoids in oral fluid specimens from individuals participating in controlled cannabis and Sativex (50% THC and 50% CBD) administration studies, and during cannabis withdrawal

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Forensic Sci Int 2010 **196** (1-3) 78

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

11-Nor-Δ^{9}-tetrahydrocannabinol-9-carboxylic acid ethyl ester (THC-COOEt) might be assumed to be a mixed metabolite produced following the combined consumption of cannabinoids and alcohol. To investigate this hypothesis, THC-COOEt and its deuterated analogue D_3-THC-COOEt were synthesized as reference substance and internal standard from the corresponding carboxylic acids and diazoethane. Procedures were developed for the sensitive detection of THC-COOEt in plasma and hair based on gas chromatography-electron impact mass spectrometry after silylation with *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide. Also, gas chromatography-negative chemical ionization mass spectrometry (GC-NCI-MS) as well as tandem mass spectrometry (GC-NCI-MS-MS) methods were developed for use after derivatization with pentafluoropropionyl anhydride. The protocols were employed for analysis of THC-COOEt in plasma samples from 22 drink driving cases which contained both ethanol (0.30-2.16 mg/g) and THC-COOH (15-252 ng/ml). They were also used to analyse 12 hair samples from drug fatalities which were both positive for THC (0.09-2.04 ng/mg) and fatty acid ethyl esters as markers of chronic alcohol abuse (0.70-6.3 ng/mg). In none of these samples was THC-COOEt found with limits of detection of 0.3 ng/ml in plasma and 2 pg/mg in hair in 11 samples using GC-NCI-MS and 0.2 pg/mg in one sample using GC-NCI-MS. Consequently, the use of THC-COOEt as a marker for combined cannabis and alcohol consumption was not accomplished

11 Narcotics

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Anal Bioanal Chem 2010 **396** (8) 3059

Simultaneous quantitation of morphine, 6-acetylmorphine, codeine, 6-acetylcodeine and tramadol in hair using mixed-mode solid-phase extraction and gas chromatography-mass spectrometry

The qualitative and quantitative analysis of several opiates (morphine, 6-acetylmorphine, codeine, 6-acetylcodeine) and tramadol in hair has been accomplished by the development and validation of a simple approach. Analytes were extracted from within the matrix *via* an overnight incubation with methanol at 65 ^{o}C. Subsequently, samples were purified by mixed-mode solid-phase extraction. Extracts were derivatized with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide with 5% trimethylchlorosilane and analysis was performed by gas chromatography-mass spectrometry in the selected ion monitoring mode. The procedure was linear from 0.05 (lower limit of quantitation) to

50 ng/mg (40 ng/mg for tramadol), with correlation coefficients greater than 0.99 for all analytes, achieving the limits proposed by the Society of Hair Testing for the detection of these substances in hair (0.2 ng/mg). Intra- and interday precision and trueness which complied with the criteria normally accepted in bioanalytical method validation. The sample cleanup step resulted in a mean efficiency higher than 90% for all analytes. Furthermore, under these incubation conditions, 6-acetylmorphine did not significantly hydrolyze to morphine. The suggested approach may be successfully applied in the determination of these analytes in a hair matrix. It is suitable for use in routine analysis for forensic purposes

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Anal Bioanal Chem 2010 **396** (7) 2533
Design of experiments, a powerful tool for method development in forensic toxicology: Application to the optimization of urinary morphine 3-glucuronide acid hydrolysis
A study has been undertake to optimize method development in the field of forensic toxicology using urinary morphine 3-glucuronide acid hydrolysis as an illustration. Urine samples were subjected to liquid-liquid extraction (ToxiTubes® A) for morphine and an internal standard of the trideuterated analogue and the analytes derivatized by silylation. Analysis was performed by gas chromatography-mass spectrometry in the selected ion monitoring mode. The peak area ratio (morphine-to-internal standard) was employed for the response. An investiagetion was performed of the independent variables that might influence the acid hydrolysis, including temperature (range 70-130 °C), acid volume (range 500-1,000 µl) and time (range 15-90 min). A 2^3 full factorial design for the screening and a response surface methodology, including a central composite design for optimization, were employed. The factors which affected the response were primarily temperature and its interaction both with time and acid volume. Application of a multiple regression analysis was applied to the experimental data and a second-order polynomial equation was produced. Optimal predicted conditions for morphine 3-glucuronide acid hydrolysis were 115 °C, 38 min and 500 µl for temperature, time and acid volume, respectively. By employing design of experiments rather than an approach of one factor at a time, optimum combination of all factor values were achieved and this facilitated production of the best data, simultaneously optimizing resources. This approach saves both time and money. Other approaches are generally more time-consuming and laborious. In addition, they do not take into account the interactions between factors

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Anal Bioanal Chem 2010 **396** (7) 2449
Analysis of ketamine and norketamine in hair samples using molecularly imprinted solid-phase extraction (MISPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS)
Ketamine is a licensed anaesthetic that is widely abused as a recreational drug. A solid-phase extraction protocol to isolate ketamine and norketamine from human hair extracts prior to LC-MS/MS analysis has been developed. It employs the synthesis of an anti-ketamine molecularly imprinted polymer (MIP) for use as a sorbent. Under optimised conditions, the MIP was capable of selectively rebinding ketamine with an apparent binding capacity of 0.13 µg ketamine/mg polymer. When 10 mg hair were analysed, the limit of detection (LOD) and lower limit of quantification (LLOQ) for both ketamine and norketamine were 0.1 ng/mg hair and 0.2 ng/mg hair, respectively. The method was linear from 0.1 to 10 ng/mg hair, with correlation coefficients (R^2) of better than 0.99 for both ketamine and norketamine. Recoveries of ketamine and norketamine from hair samples spiked at a concentration of 50 ng/mg were 86% and 88%, respectively. The method showed good intra- and interday precisions (< 5%) for both analytes. Minimal matrix effects were noted during the LC-MS/MS analysis of ketamine (ion suppression -6.8%) and norketamine (ion enhancement +0.2%). In respect of forensic case samples,it was determined that the procedure successfully detected ketamine and norketamine concentrations in hair samples at concentrations ranging from 0.2 to 5.7 ng/mg and from 0.1 to 1.2 ng/mg, respectively

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Forensic Sci Int 2010 **196** (1-3) 51
Interpretation of hair findings in children after methadone poisoning
Whereas methadone is not licensed for use in children it may be used for the management of neonatal opiate withdrawal syndrome. Over the past 2 years, Lab ChemTox has been asked to test several hair samples for methadone and its major metabolite, EDDP. Samples were obtained either from children that were admitted to hospital unconscious and where methadone had already been identified in a body fluid (4 cases) or where the children were deceased and evidence of methadone overdosage having already been established (2 cases). In all instances, segmental analysis revealed approximately the same amount of drug along the length of the hair. Consequently, contamination was considered as an issue and interpretation of the results was a challenge that deserves particular attention. Following decontamination with dichloromethane and segmentation the hair was cut into small pieces, incubated overnight at 40 °C, liquid-liquid extracted and analysed with LC-MS/MS, using 2 transitions per analyte. The LOQ for both methadone and EDDP was 10 pg/mg. In the first series involving children admitted to hospital, the following results were obtained: Case 1: 4 x 1 cm section, methadone at 0.05-0.08 ng/mg, no EDDP detected. Case 2: 4 x 1 cm section, methadone at 0.13-0.15 ng/mg, EDDP at 0.02 ng/mg. Case 3: 3 x 1.5 cm section, methadone at 0.07-0.09 ng/mg, EDDP at 0.01-0.03 ng/mg. Case 4: 6 x 2 cm section, methadone at 0.06-0.13 ng/mg, EDDP at 0.02-0.03 ng/mg. The following concentrations were obtained from the children who had died following a methadone overdose: Case 5: 2 x 2 cm section, methadone at 0.53-0.58 ng/mg, no EDDP detected. Case 6: 4 x 1 cm section, methadone at 0.44-0.77 ng/mg, EDDP at 0.04-0.06 ng/mg. It is notewrthy that all these concentrations are low in comparison with those observed in adults on methadone maintenance therapy. However, more surprisingly, is the relative homogenous concentrations along the hair locks in each specific case. This raises issues regarding the possibility that contamination could have occurred prior to sampling and makes it difficult to determine the possibility of repeated methadone exposure in the months prior the incidents. In such instances, it was impossible to conclude that the children were deliberately administered methadone. The results might be taken to indicate that the children were in an environment where methadone was being used and where the drug was not being handled and stored with appropriate care. The homogenous concentrations found on segmental analyses might suggest external contamination. This may have arisen not only from direct contamination with the drug but also *via* body fluids at the post mortem or from sweat produced close to the time of the incident. Therefore, it is concluded that a single determination should not be employed firmly to determine long-term exposure to a drug

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Forensic Sci Int 2010 **196** (1-3) 64
Solid-phase microextraction for the detection of codeine, morphine and 6-monoacetylmorphine in human hair by gas chromatography-mass spectrometry
Investigation of opiates in hair remains difficult due to the scarcity of hair sample and low drug concentrations. Therefore, a sensitive method utilizing headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) for the detection of three principle opiates (codeine, morphine, and 6-acetylmorphine) has been developed. Experimental conditions for HS-SPME and GC-MS were systematically optimized to produce the sensitive analytical procedure described. Opiates were extracted from adult hair with methanol under agitation. The methanolic extract was then decanted into SPME autosampler vials, where deuterated standards of each of the 3 opiates were added at a concentration of 2 ng/mg. Samples were dried under N_2, derivatized, and analyzed with HS-SPME coupled with GC/MS. Preliminary results suggest detection limits for these 3 opiates are superior to that reported in the literature with an LOQ of 0.01 ng/mg for morphine and 6-acetylmorphine and 0.005 ng/mg for codeine. Linearity was clearly demonstrated between 0.01 ng/mg and 5 ng/mg for each opiate, with R^2 above 0.992. The robustness of the approach was found to be acceptable as inter-day and intra-day precision fell below 15% for each opiate analyzed. In comparison with conventional procedures, this technique for opiates is fast, simple, and accurate and has a sensitivity and specificity necessary in forensic and clinical toxicology

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Am J Clin Pathol 2010 **133** (3) 447
Postmortem redistribution of fentanyl in blood
Postmortem specimens were collected from femoral blood (FB), heart blood (HB), heart tissue, liver tissue, and skeletal muscle of 20 medical examiner cases in order to analyze fentanyl concentrations. Included was a subset of 7 cases whereby FB was measured at 2 postmortem intervals, shortly after death (FB1) and at autopsy (FB2). Mean collection times of FB1 and FB2 after death were 4.0 and 21.6 hours, respectively. Fentanyl concentrations for FB1 and FB2 ranged from not detectable to 14.6 µg/l (mean, 4.6 µg/l) and 2.0 to 52.5 µg/l (mean, 17.3 µg/l), respectively. Corresponding mean HB, liver tissue, and heart tissue fentanyl concentrations were 29.8 µg/l, 109.7 mg/kg, and 103.4 mg/kg, respectively. The fentanyl HB/FB1 ratio (mean, 8.39) was higher when compared with the corresponding HB/FB2 ratio (mean, 3.48). Data indicate that postmortem redistribution of fentanyl might occur for FB

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J Chromatogr A 2010 **1217** (10) 1688
Issues pertaining to the analysis of buprenorphine and its metabolites by gas chromatography-mass spectrometry
Buprenorphine (B) as an agent for treating heroin addiction continues to gain acceptance as "substitution therapy". This policy has recently been implemented in Taiwan. To facilitate the implementation of analytical tests to support compliance monitoring and pharmacokinetic/pharmacogenetic studies a low cost and highly sensitive immunoassay (IA) is available. In addition this approach may be complemented by gas chromatography-mass spectrometry (GC-MS). Parameters pivotal to GC-MS analysis of B and norbuprenorphine (NB) (free and as glucuronides), including extraction, hydrolysis, derivatization, and quantitation approaches were examined. Data from GC-MS were compared with those produced by IA and two types of liquid

chromatography-tandem mass spectrometry (LC-MS/MS) procedures. Commercial solid-phase extraction apparatus, highly efficient for recovering all metabolites, may not be suitable for the analysis of free B and NB. Acetyl-derivatization products produce the most favorable chromatographic, ion intensity and cross-contribution characteristics necessary for GC-MS analysis. Evaluation of IA, GC-MS, and LC-MS/MS data obtained in three laboratories has demonstrated that the 2-aliquot GC-MS protocol is effective for the determination of free B and NB and their glucuronides

12 Forensics

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Forensic Sci Int 2010 **197** (1-3) 85
Increased cannabinoids concentrations found in specimens from fatal aviation accidents between 1997 and 2006
A 1.5-fold increase in the Δ^9-tetrahydrocannabinol (THC) content of street cannabis seizures from 1997 to 2001 *versus* 2002 to 2006 has been reported by the National Institute on Drug Abuse (NIDA) and the Office of National Drug Control Policy (ONDCP). A study was conducted to compare the changes, over those periods, in blood and urine cannabinoid concentrations corresponding with the potency of THC increases in the cannabis plant. Cannabinoids were screened using radioimmunoassay (RIA) for blood and fluorescence polarization immunoassay (FPIA) for urine. Confirmation employed GC/MS. Of 2769 (3.4%) individuals tested over the period 1997 through 2006, 95 individuals were found to be using cannabis. Other drugs causing impairment were found in 38% of the cannabinoids-positive individuals. The mean concentration of THC in blood for 1997-2001 was 2.7 ng/ml; for 2002-2006, it was 7.2 ng/ml, a 2.7-fold increase in the mean THC concentration of specimens from aviation fatalities, compared to a 1.5-fold increase in cannabis potency reported by the NIDA and ONDCP. The mean age for cannabis users was 40 years (range 18-72) for aviation fatalities. Blood and urine specimens testing negative for cannabinoids from aviation fatalities were from individuals where the mean age was 50 years (range 14-92). More than half of the fatalities tested were 50 years or older. However, 80% of the positive cannabis users were under 50. Consequently, members of the transportation industry, government regulators, and the general public should be made aware of the increased potential for impairment from the use of high-potency cannabis now available

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Forensic Sci Int 2010 **196** (1-3) 14
A tendency for re-offending in drug-facilitated crime
Described are three cases which illustrate a return to DFC following periods of inactivity. All offences occurred in Paris and its suburbs. In each of the instances there were two distinct periods of activity by offenders with 2, 8 and 22 victims attributed to each of the criminals. Cut hair was decontaminated and 20 mg was employed with 100 pg/mg of clonazepam-d_4 added as internal standard. Extraction of hair was performed with CH_2Cl_2/ether after incubation overnight at 56 ºC in pH 7.6 buffer. Extractions were also performed on blood and urine using Toxi-tube A with 5 ng/ml of clonazepam-d_4. Residues were analyzed by LC-ESI-MS/MS. Calibration curves in blood and urine (0.5-500 ng/ml) were prepared by spiking aliquots of blank fluids ($r^2 > 0.9816$ for all drugs). LOD in body fluids ranged 0.5-10 ng/ml. Calibration curves for hair (0.5-100 pg/mg) were prepared by spiking aliquots of blank hair ($r^2 > 0.9877$ for all drugs). LOD in hair ranged 0.5-5 pg/mg. In the first case, two young women were raped at an interval of approximately 1 year between the incidents. Lorazepam (< 2 pg/mg) was detected in hair obtained from the first victim, and zolpidem (19 pg/mg) in hair of the second one. The offender was imprisoned between the two offences. The second offender approached a total of 8 men and women who were aged over 50 years. The offender was incarcerated between the two series of respectively 3 and 5 victims. Zopiclone was detected in victims' hair (n = 7) at concentrations 13-42 pg/mg. The third offender stole thousands of Euros with credit cards taken from 22 different wealthy victims. He employed a cocktail of up to 6 drugs (flunitrazepam, clonazepam, doxylamine, cyamemazine, zolpidem and lorazepam). Drugs were detected in victims' hair (n = 18) at concentrations in the range 1-81 pg/mg for all drugs. Between the two series (of respectively 4 and 16 crimes) the offender was imprisoned for 6 months, and then police spent 6 months searching for him while he was under judiciary control prior to his judgment. Segmental hair analysis facilitates retrospective information on drug exposure It should be considered in the investigation of drug-expedited crimes not only to prove single exposure but also when there has been any appreciable delay in samples being obtained for investigation. In 56% cases reported in this paper, a long time period elapsed between offences and the opportunity to obtain samples. Therefore, analysis hair analysis was considered the only viable matrix to investigate the possibility of drug facilitation of the crimes. Our experience demonstrates that the incidence of re-offending in DFC after a period of inactivity (often due to imprisonment) may be of concern, notably in big cities

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Forensic Sci Int 2010 **197** (1-3) 89
A chloroform-related death: Analytical and forensic aspects
Chloroform is still encountered on occasions in clinical and forensic toxicology. Therefore, knowledge of any problems presented in the detection and quantification of this compound in biological specimens are necessary. This paper reviews the available documentation on this topic in the context of a chloroform-related death. Early one morning in February 1999 a 34-year-old female was found dead fully clothed on a path near to a neighbour's garden. Amphetamine intoxication combined with hypothermia was determined to be the cause of the death in the absence of any other identifiable cause. However, a subsequent investigation 17 months later revealed a blood chloroform concentration of 31 mg/l and the cause of death was now attributed to chloroform poisoning and a murder trial ensued. The indictment specified forced inhalation as the route of exposure. Thirty-eight months after collection, the liver chloroform concentration was measured at 1064 mg/kg. Autopsy findings included a gastritis but no evidence of injury to the inside of the mouth and oesophagus therby excluded the possibility of ingestion of a toxic dose of chloroform. The explanation for the high liver concentration was proposed that the liver had concentrated chloroform from blood after death against a concentration gradient. At appeal against conviction 7 years later, the conviction was quashed. It was discovered that the liver concentration should have been reported at trial as 1 mg/kg. Eighty-six months after collection, chloroform found in the stomach contents (162 mg/kg) was irrefutable evidence that some, if not all, had been ingested. Screening for volatile poisons must always be considered if a cause of death is not immediately clear. This particularly applies to young people and in known substance abusers. Should an unstable or volatile analyte be suspected, then sample collection, transport, and storage must be performed with analysis in mind. Quantitative analysis of all available specimens must commence immediately if the presence of an unstable analyte is confirmed, if the cause of death is in doubt or if prosecution is possible. Chloroform requires special precautions. Headspace analysis should be performed at 35 ºC to exclude the possibility of artefactual formation from trichloroacetic acid. Precautions to preclude cross-contamination of biological samples in the laboratory with a volatile substance must be taken. Interpretation of analytical results must take account of the widespread presence of chloroform in the environment. In addition, the toxicity of chloroform varies greatly depending on the circumstances and intensity of exposure

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Forensic Sci Int 2010 **197** (1-3) e1
Two cases of lysergamide intoxication by ingestion of seeds from Hawaiian baby woodrose
Seeds from *Argyreia nervosa* (Hawaiian baby woodrose) contain psychotropic compounds and the principal constituent is lysergamide (LSA). Two cases of human consumption were investigated whereby one resulted in a fatality due to falling from a building and one surviving witness. Analysis of urine samples for LSA was by mixed-mode cation exchange solid-phase extraction and quantification by ultra performance liquid chromatography-time of flight mass spectrometry (UPLC-ToF/MS). LSA concentrations were determined by UPLC-ToF/MS to be 4.9 µg/l in blood and 1.0mg/l in urine in the dead person and 1.8 µg/l in blood and 0.50mg/l in urine in the survivor. These analytical findings were found to agree with the case story, which indicated that seeds had been ingested and also noted psychological reactions, i.e. the will to jump out of the window. In addition, the deceased had 22 µg/l THC in blood, 0.71 g/l ethanol in blood and 1.0 g/l ethanol in vitreous humor. Other analytes originating from the seeds of *A. nervosa*, i.e. LSA, ergonovine, lysergic acid α-hydroxyethylamide were also identified in the biological samples. The 2-hydroxy-3-oxo metabolites of LSA and ergonovine were identified in the urine sample of the deceased

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Anal Chem 2009 **81** (21) 9002
Development of an information-rich LC-MS/MS database for the analysis of drugs in postmortem specimens
Several apparatus configurations of liquid chromatography-tandem mass spectrometry (LC-MS/MS) are available from manufacturers. Consequently, they have been extensively studied for comprehensive drug screening and confirmation. An LC-MS/MS database, including 780 drug and toxic compounds, has been constructed. It contains information-rich MS/MS spectra produced with a novel fragmentation approach incorporating voltage ramping and broadened mass window for activation. Resultant spectra are rich in high- and low-mass fragment ions which are highly effective for matching and proven reproducible over a 6 month test period. When linked with effective sample preparation procedures, the database-searching process markedly improved the identification of drugs in postmortem specimens by the LC-electrospray ionization (ESI)-MS/MS technology. The procedure has significantly enhanced the efficiency of the author's routine laboratory operation that was previously based on a two-step procedure of fluorescence polarization immunoassay (FPIA) and gas chromatography/mass spectrometry (GC/MS)

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Clin Chim Acta 2010 **411** (7-8) 601

Elevated lactate in ethylene glycol poisoning: True or false?
An increasing number of reports have cited a false elevation of lactate in ethylene glycol poisoning. Two cases of ethylene glycol poisoning with very high blood lactate concentrations on ABL blood gas analyzers have been investigated. Patient plasma lactate concentrations were measured on different apparatus in addition to the ABL analyzer. Serum ethylene glycol and glycolic acid were also measured. Lactate values were determined from samples spiked with various amounts of glycolic acid. All the chemistry instruments produced similar lactate results compared to that by ABL analyzer for Case 1 whereas in Case 2, the lactate on the ABL was dramatically elevated compared with that from all the chemistry analyzers. No glycolic acid detected in Case 1 but elevated glycolic acid was obtained in Case 2. Elevated concentrations of glycolic acid resulted in a significant positive interference on lactate measurements on the ABL analyzer but not on other apparatus. A false increase of blood lactate by blood gas analyzers might occur but a true increase of lactate might also be observed in ethylene glycol poisoning. It is essential that elevated lactate concentrations on blood gas analyzers be verified by a chemistry analyzer in the differential diagnosis of ethylene glycol poisoning

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Forensic Sci Int 2010 **196** (1-3) 128

Heroin markers in hair of a narcotic police officer: Active or passive exposure?
An arrest and investigation on the charges of drugs of abuse trafficking were brought against a couple consisting of a police officer (46-year-old man) and a clerk (37-year-old woman) in March 2007. It was alleged that they were exploiting their administrative positions to make money from the resale of seized drugs. A request was received to analyse their hair for drugs of abuse and both tested positive for heroin by GC-MS. Subsequently, a request was made to analyse hair from 1 other police officers of the same unit. A comparison was requires because it had been suggested that external contamination had produced. the positive results. The object of the analyses was to demonstrate that passive contamination of persons dealing every day with drugs of abuse with minimal caution and hygiene was impossible. Consequently, levels found in the hair of the arrested subjects was as a result to personal abuse. Irrespective of the compound, hair from all of the narcotics team tested negative

13 Alcohol

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Forensic Sci Int 2010 **196** (1-3) 3

Validation of a headspace solid-phase microextraction-GC-MS/MS for the determination of ethyl glucuronide in hair according to forensic guidelines
Hair is a useful matrix for chronic alcohol abuse control because of the typical wide window of detection and allows evaluation of alcohol consumption in different periods through the use of segments. The analysis of ethyl glucuronide (EtG) in hair is often the only diagnostic parameter of choice for alcohol abuse when other clinical parameters such as ALT, AST, γGT and CDT (asialotransferrin and disialotransferrin) are in the normal range and EtG in urine negative. The development of an analytical procedure for EtG in hair is described together with its optimization and validation based on extraction with water, clean-up by solid phase extraction (SPE), derivatization with heptafluorobutyric anhydride and headspace solid-phase microextraction (HS-SPME) in combination with GC-MS/MS in accordance with forensic guidelines. The linearity of the EtG assay was proven over the range from 2.8 to 1000 pg/mg hair, with a coefficient of determination (r^2) above 0.999. The LLOQ was 2.8 pg/mg and the LLOD was 0.6 pg/mg. An error profile calculated according to the "Guide to the Expression of Uncertainty in Measurement" (GUM) at 99% confidence intervals for the range 5-750 pg/mg hair did not exceed 10%. This range corresponds with greater than 98% of the positive samples analysed

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Anal Bioanal Chem 2010 **396** (7) 2441

A fully validated high-performance liquid chromatography-tandem mass spectrometry method for the determination of ethyl glucuronide in hair for the proof of strict alcohol abstinence
The detection of chronic and past drug consumption has benefitted from the ananlysis of an important new matrix; hair. In this respect, the salient compounds have been the metabolites ethyl glucuronide and fatty acid ethyl esters. Recently, new requirements have been established for the use of EtG as an abstinence test (c EtG < 7 pg/mg) as well as for heavy-drinking detection (c EtG > 30 pg/mg). In order to perform abstinence tests, a sensitive LC-MS/MS approach has been developed and fully validated in accordance with the guidelines of forensic toxicology. The nine-point calibration curve exhibited linearity over the range of concentrations from 2-1,000 pg/mg. Detection and quantification limits were 1 and 4 pg/mg respectively. The intra- and inter-day precision and accuracy were always greater than 20%. The validated protocol has successfully been employed for abstinence tests and to analyze hair samples from persons in withdrawal treatment. Concentrations between < LOQ and 400 pg/mg were determined. In some instances, interfering peaks confounded the quantification to some extent. Modified chromatographic conditions help substantiate critical data, particularly where the determined EtG concentration was close to a cut-off value

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Anal Bioanal Chem 2010 **396** (7) 2469

Quantification of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in meconium from newborns for detection of alcohol abuse in a maternal health evaluation study
A maternal health evaluation study has been conducted to detect gestational alcohol consumption. Meconium samples (n = 602) were analysed for fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG). FAEE and the total levels of ethyl palmitate, ethyl linoleate, ethyl oleate, and ethyl stearate (cut-off of 500 ng/g was applied for interpretation) were analysed by a validated headspace solid phase microextraction procedure in combination with GC-MS. Analysis of EtG consisted of 30 min. extraction with methanol/water (1:1, v/v), evaporation of methanol, filtration of the aqueous solution through a cellulose filter and injection into LC-MS-MS. A sample of 10-20 mg meconium was employed with D_5-EtG as internal standard. The limits of detection and quantification for EtG were 10 and 30 ng/g, the recovery 86.6 to 106.4% and the standard deviation of the concentrations ranged from 13% at 37 ng/g to 5% at 46,700 ng/g (N = 6). FAEE above the cut-off were found in 43 cases (7.1%) with cumulative concentrations between 507 and 22,580 ng/g and with one outlier of about 150,000 ng/g (EtG not detected). EtG was detected in 97 cases (16.3%) and concentrations between LOD and 10,200 ng/g with another outlier of 82,000 ng/g (FAEE 10,500 ng/g). Optimal agreement between the two markers was obtained with a cut-off for EtG of 274 ng/g. Of 547 cases were both FAEE- and EtG-negative, 33 cases were both FAEE- and EtG-positive, nine cases were FAEE-positive and EtG-negative, and seven cases were FAEE-negative and EtG-positive. Differences in physical, chemical, and biochemical properties and in the pharmacokinetic behavior are possible causes of the contradictory cases. Of the 602 cases, none reported serious alcohol consumption and no evidence for gestational ethanol exposure was noted in the medical examinations of the newborns. Therefore, the combined use of FAEE and EtG in meconium as biomarkers of fetal alcohol exposure improves the accuracy of the interpretation and helps circumvent positive and false-negative results

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Ann Emerg Med 2010 **55** (2) 198

Breath alcohol analyzer mistakes methanol poisoning for alcohol intoxication
The detection of ethanol in motorists and others suspected of public intoxication may be achieved by means of breath alcohol analyzers. However, a point of concern is their ability to detect interfering substances that may falsely increase the ethanol reading. A 47-year-old-man was found in a public park, behaving intoxicated. A breath analyzer test (Intoxilyzer 5000EN) measured 0.288 g/210 litres breath ethanol without an interferent being noted. Once in the emergency department, the patient admitted to imbibing HEET Gas-Line antifreeze, which contains 99% methanol. Two to three hours following ingestion, serum and urine toxicology screen results were negative for ethanol and multiple other substances. His serum methanol concentration was 589 mg/dl, serum osmolality 503 mOsm/kg, osmolar gap 193 mOsm/kg, and anion gap 17 mmol/l. He was treated with intravenous ethanol, fomepizole, and hemodialysis without complication. This is reported as a unique clinical case where a breath alcohol analyzer falsely interpreted methanol as ethanol. Intoxilyzer devices have been shown to report some substances (acetone) as interferents in humans but not methanol. Consequently, methanol may be interpreted as ethanol by a commonly used breath alcohol analyzer. This may result in delayed diagnosis or misdiagnosis and subsequent methanol toxicity if antidotal treatment is not administered in a timely manner.

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Int J Legal Med 2010 **124** (2) 161

A novel and an effective analytical approach for the LC-MS determination of ethyl glucuronide and ethyl sulfate in urine
The simultaneous determination of ethyl glucuronide (EtG) and ethyl sulfate (EtS) in urine was achieved by the development of an alternative liquid chromatography-mass spectrometry (LC-MS) method based on no discharge (ND) atmospheric pressure chemical ionization (APCI) in negative ion conditions. Abundant $[M-H]^-$ species of EtG and EtS were produced, facilitating limits of quantification to 0.1 μg/ml for both analytes. Accuracy, and precision were comparable to those cited in the literature. Furthermore, the LC-ND-APCI-MS procedure proved to be reliable. It required little maintenance even when high throughput analyses (i.e., 6,000 samples per year) were necessary

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Anal Bioanal Chem 2010 **396** (7) 2415

Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS

Biomarkers for alcohol consumption are currently under investigation. One such substance is phosphatidylethanol (PEth), an abnormal phospholipid carrying two fatty acid chains. It is only produced in the presence of ethanol *via* the action of phospholipase D (PLD). Previously, a technique for the analysis of PEth has included high-performance liquid chromatography (HPLC) coupled to an evaporative light scattering detector (ELSD) but this is unspecific for the different homologues. More recent and improved procedures utilize time of flight mass spectrometry (TOF-MS) and tandem mass spectrometry (MS/MS). This research was performed to identify as many homologues of PEth as possible. Subsequently, a screening procedure using multiple-reaction monitoring (MRM) for the identified homologues has been established. In this study, autopsy blood samples collected from heavy drinkers were employed. An internal standard of phosphatidylpropanol 16:0/18:1 was added to the blood samples before liquid-liquid extraction using borate buffer (pH 9), 2-propanol and n-hexane. Following evaporation, samples were redissolved in the mobile phase and injected into the LC-MS/MS system. Substances were separated on a Luna Phenyl Hexyl column (50 mm x 2 mm, 3 µm) by gradient elution, using 2 mM ammonium acetate and methanol/acetone (95/5; v/v). A total of 48 homologues of PEth could be identified by using precursor ion and enhanced product ion scans (EPI)

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Int J Legal Med 2010 **124** (2) 143

Practical use of ethyl glucuronide and ethyl sulfate in postmortem cases as markers of antemortem alcohol ingestion

Postmortem blood ethanol concentrations may reflect either antemortem ingestion or postmortem synthesis of alcohol. Nonoxidative ethanol metabolite ethyl glucuronide (EtG) has been proposed as a marker of antemortem ingestion of alcohol. However, EtG might be degraded postmortem and this might make interpretation difficult. To date, the published articles concern EtG only. Another nonoxidative metabolite is more stable ethyl sulfate (EtS). Therefore, it has been included in this study. Material from 36 deaths where postmortem formation of ethanol was suspected was investigated. Both EtG and EtS were measured in blood and urine to facilitate interpretation. EtG and EtS were positive in the body fluids analyzed in 19 cases. The median concentration of EtG and EtS in blood was 0.4 (range 0.1-23.2) and 0.9 mg/l (range 0.04-7.9), respectively. Whereas the median concentration of EtG and EtS in urine was 35.9 (range 1.0-182) and 8.5 mg/l (range 0.3-99), respectively. In 16 other cases, there was no trace of EtG or EtS in the specimens analyzed. In one case, there was inconsistency between the results of EtG and EtS; they were both positive in urine, while only EtS was positive in blood. Therefore, in 36 cases according to EtG and EtS results, antemortem ingestion of alcohol was probable in 19 and unlikely in 16. In the other case, the interpretation was more difficult. A possible interpretation might be postmortem degradation of EtG in blood

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Forensic Sci Int 2010 **196** (1-3) 74

Ethyl glucuronide and ethyl sulfate in meconium and hair-potential biomarkers of intrauterine exposure to ethanol

Alcohol consumption in a cohort of 99 mother-infant dyads, 49 coming from the Arcispedale of Reggio Emilia (Italy) and 50 from the Hospital del Mar of Barcelona (Spain) was investigated in respect of *in utero* exposure. Ethyl glucuronide (EtG) and ethyl sulfate (EtS) concentrations were analyzed in meconium and in maternal and neonatal hair as potential markers of intrauterine exposure to ethanol together with meconium fatty acid ethyl esters (FAEEs). Liquid chromatography-tandem mass spectrometry was employed to measure FAEEs, EtG and EtS. Head space-solid phase microextraction-gas chromatography-mass spectrometry was used to analyze HEtG and HFAEEs in hair samples from mothers and their newborns. Eighty-two meconium samples (82.8%) tested positive for EtG, 19 (19.2%) for EtS while 22 (22.2%) showed FAEEs levels higher than 2 nmol/g, the limit used to distinguish daily maternal alcohol consumption during pregnancy from occasional or no use. Whereas EtG and EtS in meconium did not correlate with total FAEEs concentration, a good correlation between EtG, EtS and ethyl stearate was noted. Furthermore, EtG correlated well with ethyl palmitoleate and EtS with ethyl laurate, myristate and linolenate. Neither maternal nor neonatal hair appears satisfactory matrices to measure gestational ethanol consumption and subsequent fetal exposure in these mother-infant dyads. Data indicate that meconium is so far the best matrix in evaluating *in utero* exposure to ethanol, with EtG and EtS being potentially good alternative biomarkers to FAEEs

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Accredit Qual Assur 2010 **15** (3) 141

Ethanol/water solutions as certified reference materials for breath alcohol analyzer calibration

A series of large volume ethanol in water certified reference materials (CRMs), primarily developed for the calibration of evidential breath alcohol analyzers in Germany has been distributed by the Federal Institute for Materials Research and Testing (BAM), Germany. The certified parameter is the ethanol mass concentration at 20 ºC. When employed in a wet bath simulator, the solutions deliver gas samples that meet the parameters by issued the Organization of Legal Metrology for calibration of breathalyzers. Solutions were prepared gravimetrically by spiking of ethanol into water in single 5 litre units. A complete uncertainty budget for the preparation procedure has been established. The purity of the commercial ethanol stock solution was determined to be the main source of uncertainty. A robust high-precision gas chromatography, with flame-ionization detection method for ethanol determination in aqueous samples was developed and validated. This was employed to confirm stability and homogeneity and to verify the gravimetric mass concentration of the CRMs. The good performance of this approach has been demonstrated in several international comparisons organized by the Consultative Committee for Amount of Substance-Metrology in Chemistry at the International Bureau of Weights and Measures

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Forensic Sci Int 2010 **196** (1-3) 101

Combined use of fatty acid ethyl esters and ethyl glucuronide in hair for diagnosis of alcohol abuse: Interpretation and advantages

Diagnoses of chronically excessive alcohol abuse has been investigated in 174 hair samples from driving ability examination, workplace testing and child custody cases for family courts and evaluated with respect to the basics of interpretation. It has employed measurement of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG). Using the cut-off values of 0.50 ng/mg for FAEE and 25 pg/mg for EtG, both markers were in agreement in 75% of the cases with 103 negative and 28 positive results. There were 30 cases where FAEE was positive and EtG negative and 13 cases with FAEE negative and EtG positive. In respect of the theoretical basis of interpretation, the pharmacokinetics of FAEE and EtG is reviewed for all steps between drinking of ethanol to incorporation in hair. Particular attention is applied to the relationship between alcohol dose and concentrations in hair. It is demonstrated that the concentrations of both markers are essentially determined by the area under the ethanol concentration in blood *vs*. time curve AUC(EtOH), despite large inter-individual variations. By calculation of AUC(EtOH) on monthly basis for moderate, risky and heavy drinking, it is shown that AUC(EtOH) increases very strongly in the range between 60 and 120 g ethanol per day. This specific feature which is caused by the zero-order elimination of ethanol is a beneficial requirement for a high discrimination power of the hair testing for alcohol abuse. Considering of the different profiles of FAEE and EtG along the hair and in accordancet with a literature survey, a standardized hair segment 0-3 cm is proposed with cut-off values of 0.5 ng/mg for FAEE and 30 pg/mg for EtG. In addition, this benefits the agreement between FAEE and EtG data in the present study. A scheme for combined interpretation of FAEE and EtG is suggetsed. It employs the levels of abstinence and double the cut-offs values as criteria in addition to the cut-off values. In respect of the large variations in the relationship between ethanol dose and FAEE and EtG concentrations in hair, the combined use of both parameters strongly increases the accuracy of the diagnosis. It provides confirmation and also identification of false positive or false negative results due to biological variations or analytical errors

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Forensic Sci Int 2010 **196** (1-3) 111

Fatty acid ethyl ester concentrations in hair and self-reported alcohol consumption in 644 cases from different origin

Hair samples were obtained from 644 individuals who were mostly parents from child protection cases. They were analysed for fatty acid ethyl esters (FAEE) as biomarkers for the diagnosis of chronic alcohol abuse. Analyses were performed for ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate. These were carried out according to a validated protocol consisting of external degreasing by two times washing with *n*-heptane, extraction with a mixture of dimethylsulfoxide and *n*-heptane, separation and evaporation of the n-heptane layer, headspace solid phase microextraction of the residue after addition of phosphate buffer pH 7.6 and gas chromatography-mass spectrometry employing deuterated internal standards. For interpretation, the sum of the concentrations of the four esters C_{FAEE} was used with the cut-off's 0.5 ng/mg for the proximal scalp hair segment 0-3 cm or less and 1.0 ng/mg for scalp hair samples with a length between 3 and 6 cm and for body hair. C_{FAEE} ranged from 0.11 to 31 ng/mg (mean 1.77 ng/mg, median 0.82 ng/mg). The mean concentration ratio between the 4 esters was 8:45:38:9. Two hundred and ninety-eight cases had C_{FAEE} above the cut-off's. In 553 of the cases, self-reported drinking data were obtained. These were classified as abstinent (156), moderate consumption (252) and excessive consumption (145). Median and box-plot data plainly illustrate discrimination of these categories by C_{FAEE}. However, in the abstinent and moderate groups, consumption was frequently underreported (37 and 110 cases positive). Conversely, in the self-reported excessive drinking group, 32 cases were negative. Comparison of C_{FAEE} with carbohydrate-deficient transferrin (CDT) in 139 cases and γ-glutamyltransferase (GGT) in 136 cases demonstrated a good agreement in CDT and GGT positive cases (27/28 and 32/41). However, a large portion of the negative CDT and GGT results with positive hair test (44/100 and 48/95) might result primarily from the much shorter time window of CDT and GGT. A significant correlation was not noted between participant's weight and C_{FAEE}. Therefore, the test was not biased against physical fitness or obesity. In addition, there was no statistically significant difference between scalp hair (541 samples) and hair

from other body sites (84 samples). Therefore, FAEE in hair appeared to be a suitable analyte for the detection of excessive drinking. However, because is no proportionality between drinking amount and C_{FAEE}, further markers would increase the reliability of the interpretation

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Forensic Sci Int 2010 **196** (1-3) 121

Ethyl glucuronide determination in meconium and hair by hydrophilic interaction liquid chromatography-tandem mass spectrometry

Alcohol abuse over a long period, for example, during pregnancy or after a withdrawal treatment might be measured by the ethyl glucuronide (EtG) content of non-conventional matrices, such as hair and meconium. The analysis of EtG in meconium and hair was achieved by the development, validation and application of a new hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) procedure. For both hair and meconium, sample preparation and chromatographic separation were rigorously optimised. In the development stages, additional experiments with reversed-phase liquid chromatography were also carried out. Analyses were performed with a Phenomenex Luna HILIC column (150 mm x 3 mm, 5 µm) and a mobile phase composed by ammonium acetate 2mM and acetonitrile, in gradient. Various SPE cartridges (Oasis MAX, Oasis WAX, aminopropyl silica) and solvents were investigated in order to obtain the highest recoveries and cleanest extracts. Optimal data were produced for meconium with aminopropyl cartridges, while for hair an incubation of 16 h with 2 ml of water and acetonitrile (50/50, v/v) resulted in good results. The analytical procedure was validated for both matrices (meconium and hair) by assessing linearity, precision, accuracy, recovery and limit of quantification. Calibration curve concentrations ranged from 50 to 1200 pg/mg for meconium and from 20 to 1000 pg/mg for hair. Real meconium and hair samples were analyzed and results were consistent with those in the literature

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Ther Drug Monit 2010 **32** (2) 216

Gas chromatography tandem mass spectrometry for biomarkers of alcohol abuse in human hair

The detection of potential alcohol abuse by the measurement of fatty acid ethyl esters (FAEEs) in human hair has been assisted by the development of a novel, faster analytical technique. FAEEs are recognized metabolites of ethanol in humans and are embedded in the hair follicles during hair growth. The developed procedure has a total analysis time-including washing, extraction, concentration, separation, and detection-of less than 1 hour whereas normally used extraction procedures in the literature for these biomarkers are typically 15 hours. Analysis is achieved using gas chromatography-tandem mass spectrometry (GC-MS/MS) with a GC separation time of less than nine minutes. Using chemical ionization, mass spectrometric detection consists of selected reaction monitoring. This is generally considered to be one of the most selective and sensitive forms of mass spectrometric detection. Employing selected reaction monitoring facilitates a reduction of interferences from the hair matrix, thereby making the protocol more selective for the biomarkers. Limits of detection for each FAEE range from 0.002 to 0.030 ng/mg in hair. Through the application of this more rapid extraction approach, data indicate that this method might potentially distinguish whether a person is a heavy drinker, moderate drinker, or nondrinker. In addition, rapid analysis of hair samples might be applied to a number of different areas, such as neonatal screening, parole violations, contributing factors in the cause of death, and any other situation necessitating the establishment of chronic *versus* acute alcohol abuse.

14 Tobacco

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Anal Bioanal Chem 2010 **396** (8) 2987

Evaluation of potential breath biomarkers for active smoking: Assessment of smoking habits

Several compounds have been reported as biomarkers of degree of tobacco smoking. However, to date, no biomarker has been found to distinguish smokers from non-smokers. Standard sampling procedures necessitate relatively invasive approaches because blood or urine samples are required. A totally noninvasive approach would be to analyse volatile organic compounds in breath samples. In this respect, the use of a microtrap system coupled to gas chromatography-mass spectrometry has been found to be very effective. Participants consisted of 204 volunteers of which 100 were smokers, 104 nonsmokers, 147 females, 57 males and aged 16 to 53 years. Benzene, 2,5-dimethylfuran, toluene, *o*-xylene, and *m*- *p*-xylene were analyzed in breath samples. 2,5-Dimethylfuran was always below the limit of detection (0.005 ppbv) in the nonsmoker population and always detected in smokers independently of the smoking habits. Benzene was only useful as a biomarker for medium and heavy smokers, and its level was affected by smoking habits. In respect of the levels of xylenes and toluene, they were only different in heavy smokers and after short-term exposure. Therefore, it is clear that 2,5-dimethylfuran is a specific breath biomarker of smoking status independently of the smoking habits (e.g., short- and long-term exposure, light and heavy consumption). Consequently, this compound might be useful as a biomarker of tobacco exposure

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Anal Chim Acta 2010 **663** (1) 49

An improved headspace solid-phase microextraction method for the analysis of free-base nicotine in particulate phase of mainstream cigarette smoke

Due to procedural issues, he content of free-base nicotine in cigarette smoke is a contentious issue. Free-base nicotine in cigarette smoke was measured by an improved technique employing headspace solid-phase microextraction (HS-SPME) combined with GC/MS analysis which was developed and validated for this objective. Cigarette smoke particulate phase (PP) was collected onto a 44mm glass fiber filter pad. The pad was cut in halves with one half used to assess the concentrations of total nicotine and water. The remaining half was analyzed by HS-SPME for free-base nicotine. Several parameters were discovered to have a notable effect on the responses of free-base nicotine. These included SPME fiber type, pre-equilibrium time before HS-SPME, extraction time and temperature, PP water content, and the solvent used for the preparation of standards. It was determined that the impact of PP water content on the measurement of free-base nicotine from the smoke sample could be corrected by a water correction factor calculated when based on an experimentally determined reciprocal model. The precision of the approach was assessed with smoke samples of reference cigarettes: Canadian flue-cured monitor and Kentucky reference 2R4F. The resulting RSD values were in the 12.8-16.8% range

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Chromatographia 2010 **71** (3-4) 259

GC-MS analysis of hydrogen sulfide, carbonyl sulfide, methanethiol, carbon disulfide, methyl thiocyanate and methyl disulfide in mainstream vapor phase cigarette smoke

A procedure employing gas chromatography-mass spectrometry has been developed for the simultaneous analysis of hydrogen sulfide, carbonyl sulfide, methanethiol, carbon disulfide, methyl thiocyanate and methyl disulfide in mainstream vapor phase (MVP) cigarette smoke. Fresh MVP smoke was collected in a gas bag A 50 µl sample was injected into the GC inlet *via* an automatic six- port valve. Separation was on a CP-PoraPLOT Q column and MS was operated in SIM mode. Whereas carbonyl sulfide and carbon disulfide were found to very stable in the gas bag, hydrogen sulfide, methanethiol, methyl disulfide and methyl thiocyanate were extremely reactive and their levels increased or decreased drastically with the storage time. Therefore, there is an absolute necessity to analyze the smoke sample as quickly as possible. In order to obtain reproducible results, maintaining a precise time after the smoke collection is a vital parameter. In this work, all samples were injected within 2 min after MVP smoke was collected in the bag. Using smoke conditions of 60 ml puff of 2 s duration every 30 s, 12 brands of commercial cigarettes and Kentucky Reference 2R4F cigarettes were analyzed. Average values of three replicates of the 2R4F cigarettes were 31.6 µg/cigt hydrogen sulfide, 40.7 µg/cigt carbonyl sulfide, 25.6 µg/cigt methanethiol, 2.2 µg/cigt carbon disulfide, 23.7 µg/cigt methyl thiocyanate and 17.6 µg/cigt

Fix BV, O'Connor R, Hammond D, King B, McNeill A, Thrasher J, Boado M, Cummings KM, Yong HH, Thompson ME, Hyland A// Roswell Pk Canc Inst, Dept Hlth Behav, Elm & Carlton St, Buffalo, NY 14263, USA

Nicotine Tob Res 2010 **12** (3) 185

ITC "spit and butts" pilot study: The feasibility of collecting saliva and cigarette butt samples from smokers to evaluate policy

Large-scale epidemiological surveys have often employed clinic-based specimen collection to incorporate biological data. This might be costly and result in nonrepresentative data. Obtaining samples in a nonclinical setting provides an alternative procedure that is minimally invasive and might be incorporated into large population-based surveys. This research was performed to assess the feasibility of collecting biological data from a cohort of smokers in the International Tobacco Control (ITC) study, through the mail and in the home. In addition, whether participants were representative of the population was considered as was whether the added burden of providing biomarker samples might impact subsequent participation in a follow-up survey. Participants were requested to provide a saliva sample and five cigarette butts from cigarettes smoked on a single day, using standardized procedures. Sample collection kits were posted to a random sample of 400 daily cigarette smokers who were involved in the 2006 annual ITC Four Country (United Kingdom, United States, Canada, and Australia) telephone survey and agreed to participate in sample collection. A random sample of 179 daily smokers who took part in a face-to-face ITC survey in Mexico and Uruguay also agreed to participate in sample collection were requested to provide samples. Samples were collected from 96% of invited participants in the face-to-face surveys and 52% of participants in the telephone survey. The added burden of the sample collection was not noted to reduce survey retention rates. Participants who originally agreed to take part in the sample collection were found to be more likely to participate in the subsequent survey than participants who were not asked or declined to participate (odds ratio [OR] = 1.28; 95% CI = 1.01-1.62, p = 0.021). Furthermore, those who provided samples were also more likely to participate in the

subsequent survey than those who did not (OR = 2.78; 95% CI = 1.71-4.52, p < 0.001). Collecting saliva and cigarette butt samples from a group of smokers is possible. It provides a representative sample, and the added participant burden does not reduce subsequent survey response rates

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Talanta 2010 **81** (1-2) 650

Accelerated solvent extraction for GC-based tobacco fingerprinting and its comparison with simultaneous distillation and extraction

Chemical fingerprinting of volatile and semi-volatile components in cut tobacco has been achieved by development of an accelerated solvent extraction (ASE) approach. ASE extraction conditions including temperature, operation pressure and extraction cycles were optimized in respect of yield. The procedure was validated with repeatability, recovery and linearity. When compared with simultaneous distillation extraction (SDE), ASE provides higher extraction yields, less extraction time, lower solvent consumption and less labor time. It is more suitable for tobacco sample preparation. The ASE extract was analyzed by gas chromatography/time-of-flight mass spectrometry (GC-TOFMS). Using the NIST05 and Wiley database, 305 components with signal-to-noise ratio higher than 100 were tentatively identified. Using the developed chemical fingerprinting method, 36 cigarette samples from six cigarette brands were analyzed. Partial least squares-discriminant analysis demonstrated good discrimination of different cigarette brands. Data suggest that the ASE approach might serve as a high-throughput sample preparation technique for cigarette chemical fingerprint analysis

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J Chromatogr B 2010 **878** (9-10) 725

A novel validated procedure for the determination of nicotine, eight nicotine metabolites and two minor tobacco alkaloids in human plasma or urine by solid-phase extraction coupled with liquid chromatography-electrospray ionization-tandem mass spectrometry

The simultaneous determination of nicotine-*N*-β-D-glucuronide, cotinine-*N*-oxide, *trans*-3-hydroxycotinine, norcotinine, *trans*-nicotine-1'-oxide, cotinine, nornicotine, nicotine, anatabine, anabasine and cotinine-*N*-β-D-glucuronide in human plasma or urine has been accomplished by the development and validation of a novel validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) procedure. Target compounds and their deuterated internal standards were extracted by a solid-phase technique and analyzed by LC-MS/MS with electrospray ionization (ESI) using multiple reaction monitoring (MRM) data acquisition. Calibration curves were linear over the selected concentration ranges for each compound, with calculated coefficients of determination (R^2) of greater than 0.99. Total extraction recovery (%) was concentration dependent and ranged between 52-88% in plasma and 51-118% in urine. Limits of quantification for all compounds in plasma and urine were 1.0 ng/ml and 2.5 ng/ml, respectively, with the exception of cotinine-*N*-β-D-glucuronide, which was 50 ng/ml. Intra-day and inter-day imprecision were < or = 14% and < or = 17%, respectively. Matrix effect (%) was sufficiently minimized to < or = 19% for both matrices using the described sample preparation and extraction techniques. Target analytes were stable in both matrices for at least 3 freeze-thaw cycles, 24 h at room temperature, 24 h in the refrigerator (4 °C) and 1 week in the freezer (-20 °C). Reconstituted plasma and urine extracts were stable for at least 72 h storage in the liquid chromatography autosampler at 4 °C. Samples were obtained from nicotine-abstinent human participants as part of a pharmacokinetic study investigating biomarkers of nicotine use in plasma following controlled low dose (7 mg) transdermal nicotine delivery. The plasma procedure was successfully applied to the quantitative determination of nicotine, cotinine, *trans*-3-hydroxycotinine and *trans*-nicotine-1'-oxide. The urine procedure was employed in the monitoring of unauthorized tobacco use by clinical study participants at the time of physical examination (before enrollment) and on the pharmacokinetic study day

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Forensic Sci Int 2010 **196** (1-3) 97

Assessment of exposure to environmental tobacco smoke in young adolescents following implementation of smoke-free policy in Italy

Following recent implementation of Italian smoke free legislation, acute and chronic exposure to environmental tobacco smoke (ETS) in a cohort of young adolescents was examined using urinary cotinine and hair nicotine testing. Participants were 372 Italian young adolescents, between 10 and 16 years of age from the principal city of Sicily, Palermo. Between November 2005 and May 2006, when the legislation to ban smoking in all the enclosed places of employment (including bars, restaurants, pubs) was completely enforced, urine and hair samples were collected. An exhaustive questionnaire including sociodemographic characteristics and active and passive exposure to cigarette smoking was completed. Cotinine in urine was analyzed by radioimmunoassay and nicotine in hair by a validated GC/MS method. Based on urinary cotinine results, 2.1% and 89% of the study subjects, respectively, demonstrated non-exposure and low acute exposure to ETS. Only 1.6% presented very high exposure or a hidden active smoking habit in the recent past. Hair nicotine revealed non-exposure and low exposure to ETS in 11.8% and 65.6% of the young adolescents, respectively, taking into consideration a larger time-window. High repeated exposure, indicating active smoking in some cases was observed in 8.6% of the study subjects. An inverse relationship was noted between hair nicotine and educational level of the adolescents' parents.Following implementation of smoke-free legislation and an information campaign against smoking, a significant trend toward low exposure to ETS was noted in this cohort. There was no association between exposure to ETS and respiratory illnesses

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Chem Res Toxicol 2010 **23** (1) 66

Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography-mass spectrometry

Precancerous oral lesions and oral and pancreatic cancer are known to be a consequence of smokeless tobacco consumption. It contains at least 28 known carcinogens. Recently, the authors identified eight different polycyclic aromatic hydrocarbons (PAHs) in U.S. moist snuff. This stimulated further studies of this group of toxicants and carcinogens in smokeless tobacco products. A gas chromatography-mass spectrometry approach that allows simultaneous analysis of 23 various PAHs in smokeless tobacco after a simple two-step extraction and purification procedure has been developed. The technique resulted in coefficients of variation under 10% for most PAHs. The limits of quantitation for different PAHs varied between 0.3 and 11 ng/g tobacco, starting with a 300 mg sample. Recovery of the stable isotope-labeled internal standards averaged 87%. The procedure was employed to analyse 23 samples that included various flavors of the most popular U.S. moist snuff brands. In addition, 17 samples representing the currently marketed brands of spit-free tobacco pouches, a relatively new type of smokeless tobacco, were analysed. The total of all detected PAHs in conventional moist snuff averaged 11.6 (+/-3.7) μg/g dry weight and 20% of this was comprised of carcinogenic PAHs. Levels of PAHs in new spit-free tobacco products were far lower than those in moist snuff and the total of all detected PAHs averaged 1.3 (+/-0.28) μg/g dry weight. Therefore, PAHs are one of the most predominant groups of carcinogens in smokeless tobacco. Consequently, immediate measures are necessary by the U.S. tobacco industry to modify manufacturing processes such that the levels of these toxicants and carcinogens in U.S. moist snuff are far lower

15 Homeland Security

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Anal Chem 2010 **82** (5) 2042

Nano aptasensor for protective antigen toxin of anthrax

A highly sensitive nano aptasensor for anthrax toxin has been developed which detects the polypeptide entity, protective antigen (PA toxin) by employing PA toxin ssDNA aptamer functionalized single-walled carbon nanotubes (SWNTs). The aptamer was developed in-house by capillary electrophoresis systematic evolution of ligands by exponential enrichment (CE-SELEX) and has a dissociation constant (K_d) of 112 nM. The aptasensor displayed a wide dynamic range spanning up to 800 nM and had a detection limit of 1 nM. The sensitivity was 0.11 per nM. It could be reused six times. It proved highly selective for PA toxin and displayed no interference from human and bovine serum albumin. Therefore, it as a potential tool for rapid and point-of-care diagnosis for anthrax

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Anal Chem 2010 **82** (8) 3191

Impedance based detection of chemical warfare agent mimics using ferrocene-lysine modified carbon nanotubes

A sensor for chemical warfare agent (CWA) mimics was fabricated with a recognition layer formed by multiwalled carbon nanotubes (MWCNTs) covalently modified with a ferrocene-lysine conjugate deposited on the indium tin oxide (ITO). Electrochemical impedance spectroscopy measurements demonstrated that following addition of CWA mimic, marked changes occurred in the electrical properties of the recognition layer. This facilitated the detection of nerve agent analogues at the micromolar level. In addition, a limited sensitivity was observed toward a sulfur mustard mimic was noted. Operational parameters were optimized expedite the detection of CWAs at single frequency, thus significantly reducing acquisition time and simplifying data treatment. This represents a significant step toward the design of an affordable and "fieldable" electrochemical CWA sensor

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Anal Chem 2009 **81** (21) 8892

Detection and quantification of chemical warfare agent precursors and surrogates by selected ion flow tube mass spectrometry
The rate coefficients and branching ratios of 15 chemical warfare agent precursor and surrogate compounds which react with H_3O^+, NO^+, and O_2^+ have been quantified in a laboratory employing selected ion flow tube (SIFT) technique. Measurement of the relevant kinetic parameters for these agents has facilitated quantitative analysis utilising the SIFT-MS procedure. Thirteen of the 15 compounds investigated were shown to have real-time detection limits in the parts-per-trillion-by-volume concentration range when measured on a standard commercial Voice100 instrument. Certain compounds had detection limits below 100 parts-per-trillion-by-volume

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J Food Prot 2010 **73** (4) 695
Development of a novel immuno-PCR assay for detection of ricin in ground beef, liquid chicken egg, and milk
Food defense, the detection of foodborne contaminants and the facilitation of remediation and recovery from potential toxin-related incidents requires eliable, sensitive, and high-throughput techniques. Ricin, a lectin for the castor bean, is a protein toxin that has been previously employed for intentional contamination of foods. Methods were developed for quantification of ricin in foods using immuno-PCR (IPCR). The direct adsorption of ricin onto the wells of a microtitration plate was compared with indirect immobilization *via* a capture antibody (sandwich IPCR). The latter procedure provided far superior sensitivity. Procedures were compared for the immunoassay and PCR conducted in a single plate with a two-step protocol whereby the PCR was conducted in a second plate, following release and transfer of the DNA marker. The two-step procedure demonstrated 1,000-fold greater sensitivity for ricin detection. Therefore, this approach was employed to detect ricin in spiked samples of ground beef, chicken egg, and milk. Data were compared with those obtained from enzyme-linked immunosorbent assay (ELISA). The IPCR had a limit of detection of 10 pg/ml in chicken egg and milk samples and 100 pg/ml in ground beef extracts. Comparable ELISA results were in the 1-10 ng/ml range. Therefore, IPCR facilitates a sensitivity that is 10-fold greater in the ground beef matrix, 100-fold greater in the milk, and 1,000-fold greater in the egg matrix than the sensitivity obtained by ELISA. Further optimization of the sandwich IPCR was accomplished by comparing various antibody combinations. Of the four formats investigated, the pAb-pAb combination yielded the lowest limit of detection (10 fg/ml)

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Biosens Bioelectron 2010 **25** (7) 1566
A novel organophosphorus hydrolase-based biosensor using mesoporous carbons and carbon black for the detection of organophosphate nerve agents
A novel biosensor based on organophosphorus hydrolase has been developed which permits detection of organophosphate chemicals both as pesticides and as nerve agents. Mesoporous carbon (MC) and carbon black (CB) were employed as an anodic layer and this resulted in a marked improvement in the sensitivity of the sensor to the product of the organophosphorus hydrolase reaction, *p*-nitrophenol (PNP). The MC/CB/glass carbon (GC) layer showed an improved amperometric response relative to a carbon nanotube (CNT)-modified electrode due to the promotion of electron transfer of PNP. The well-ordered nanopores, many edge-plane-like defective sites (EDSs), and high surface area of the MC promoted sensitivity, and enabled nanomolar-range detection of the analyte paraoxon. Thus, MCs are applicable for use in real-time biosensors. Under the optimized experimental parameters, the biosensor had a detection limit of 0.12 μM (36 ppb) and a sensitivity of 198 nA/μM for paraoxon

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J Chromatogr A 2010 **1217** (17) 2887
Application of high performance liquid chromatography coupled to on-line solid-phase extraction-nuclear magnetic resonance spectroscopy for the analysis of degradation products of V-class nerve agents and nitrogen mustard
A high performance liquid chromatography procedure coupled to on-line solid-phase extraction-nuclear magnetic resonance spectroscopy (HPLC-UV-SPE-NMR) has been employed to detect and identify the degradation products of nitrogen mustard and nerve agent VX. *N,N*-dimethylaminoethanol (DMAE), *N,N*-diethylaminoethanol (DEAE), *N,N*-diisopropylaminoethanol (DIAE) and triethanolamine (TEA) were selected for analysis. To eliminate interferents and render the analytes suitable for UV detection, offline solid-phase extraction (SPE) was followed by derivatization. Following, HPLC separated analytes were trapped on on-line SPE cartridges. Subsequently they were eluted and 1H NMR and COSY spectra obtained. Overall detection limits of the LC-UV-SPE-NMR method for DMAE, DEAE, DIAE and TEA were found to be 18, 23, 25, and 32 mg/l respectively. Application of the approach to real samples was demonstrated by the analysis of samples provided during the 22nd OPCW official proficiency test. The technique produced reproducible NMR spectra without intense background signals

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J Chromatogr A 2010 **1217** (14) 2171
Headspace-trap gas chromatography-mass spectrometry for determination of sulphur mustard and related compounds in soil
Headspace-trap in combination with gas chromatography-mass spectrometry (GC-MS) has been developed for trace determination of sulphur mustard (HD) and some related cyclic sulphur compounds in soil samples. Sandy loam and silty clay loam were utilised for method optimisation. Before analysis, water saturated with sodium chloride was added to the samples, at a water to soil ratio of 1:1. A detection limit of 3 ng/g was achieved for HD, while the cyclic sulphur compounds 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane could be detected at 0.2-0.7 ng/g. The procedures were validated in the concentration range from the limit of quantification (LOQ) to one hundred times LOQ. Within assay precision at fifty times LOQ was 6.9-7.3% relative standard deviation (RSD) for determination of the cyclic sulphur compounds, and 15% RSD for determination of HD. From the two soil types, recoveries were in the range of 43-60%. The procedure necessitates very little sample preparation and thus the total time for sample handling and analysis was less than 1h. The protocol was successfully applied to the determination of cyclic sulphur compounds in a sediment sample from an old chemical munitions dumping site, known to contain HD degradation products

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Talanta 2010 **81** (1-2) 703
Amperometric immunosensor for ricin by using on graphite and carbon nanotube paste electrodes
Ricin, a lectin from castor bean, is a protein and scheduled chemical and biological warfare agent. Amperometric immunosensors were devised for the detection of ricin in water samples. Electrodes were fabricated by mixing Paraffin oil with graphite powder and multiwalled carbon nanotubes. The graphite paste electrode (CPE) and multiwalled carbon nanotubes paste electrode (MWCNTPE) were tested for their ability to detect 1-naphthol. A sandwich enzyme linked immunosorbent assay system was employed to detect ricin. The detection limit for both electrodes was compared. It was noted that the response of the amperometric sensor is proportional to the ricin concentration in both cases and is linear in the range 0.625-25 ng/ml for MWCNTPE and 2.5-25 ng/ml for CPE. The SEM showed that the MWCNTPE has revealed crevices/voids in which the antibodies may become trapped. Furthermore, spectroscopic experiments demonstrated that MWCNTPE adsorbs antibodies better than CPE. However, the high sensitivity of MWCNTPE was attributed to its superior electrochemical properties as opposed to its efficiency to adsorb antibodies

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Appl Radiat Isot 2010 **68** (4-5) 888
Fast neutron sensor for detection of explosives and chemical warfare agents
In shallow coastal sea waters, once a muition has been detected, it is necessary to esablish whether it contains explosive or chemical warfare charge. It is suggested that this be achieved by using a neutron sensor installed within a submersible. The vehicle is positioned above the object or to alongside. The neutron sensor is then inspect the object for the presence of the threat materials by using alpha particle tagged neutrons from the sealed tube d+t neutron generator

16 Workplace

Curwin BD, Hein MJ, Barr DB, Striley C// Ctr Dis Control & Prevention, NIOSH, Div Surveillance Hazard Evaluation & Field Studies, Cincinnati, Oh 45226, USA
J Expo Sci Environ Epidemiol 2010 **20** (2) 205
Comparison of immunoassay and HPLC-MS/MS used to measure urinary metabolites of atrazine, metolachlor and chlorpyrifos from farmers and non-farmers in Iowa
Fifty-one people participated in a study investigating pesticide exposure among farm families in Iowa by providing urine samples. Aliquots from the specimens were analyzed by two different labs for metabolites of atrazine (atrazine mercapturate), metolachlor (metolachlor mercapturate) and chlorpyrifos (TCP). Two different techniques were employed namely immunoassay and high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The HPLC-MS/MS methods tended to be highly specific, but are costly and time consuming. Immunoassay methods were cheaper and faster, but due to cross reactivity and matrix effects might be less sensitive. Three statistical methods were utilised to compare the two analytical methods. Each statistical method differed in how the samples which had results below the limit of detection (LOD) were treated. The first two methods involved an imputation procedure and the third method used maximum likelihood estimation (MLE). A fourth statistical method that modeled each lab separately employing MLE was applied for comparison. Immunoassay and HPLC-MS/MS procedures were moderately correlated (correlation 0.40-0.49) but the immunoassay techniques consistently produced significantly higher geometric mean (GM) estimates for each pesticide metabolite. The GM estimates for atrazine mercapturate,

metolachlor mercapturate, and TCP by immunoassay ranged from 0.16-0.98 µg/l, 0.24-0.45 µg/l and 14-14 µg/l, respectively and by HPLC-MS/MS ranged from 0.0015-0.0039 µg/l, 0.12-0.16 µg/l, and 2.9-3.0 µg/l, respectively. Immunoassays tended to be cheaper and faster than HPLC-MS/MS. However, they may result in an upward bias of urinary pesticide metabolite levels

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Forensic Sci Int 2010 **197** (1-3) e19
Occupational asphyxiation by unknown compound(s): Environmental and toxicological approach
Five previously healthy workers were found motionless inside an empty chemical tanker during a routine truck-tank washing operation. Four succumbed inside the tanker while the fifth died the following day in hospital. A rigorous environmental and toxicological protocol supported by autopsy findings was essential to clarify the cause of death because the true nature of the fatal compound(s) was unknown. Environmental results suggested that H_2S fumes arising from the liquid sulfur previously shipped in the tanks were responsible for the serial deaths. This was ratified by a simulation performed on two similar truck-tanks. Environmental findings were endorsed by toxicological analyses involving the measurement of thiosulfate, one of the main H_2S metabolites. Abnormal thiosulfate concentrations from 1.1 to 186.2 mg/kg were revealed in all post-mortem biological samples (blood, lung, liver, kidney, brain and fat). Finally, cluster analysis performed on thiosulfate body distribution contributed to establishing the time of death according to the accident scene reconstruction. This research presents valuable findings in correctly identifying the cause of death in gas asphyxiation cases by unknown compound(s)

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Analyst 2010 **135** (5) 994
Facility monitoring of toxic industrial compounds in air using an automated, fieldable, miniature mass spectrometer
A fully automated, field-applicable, miniature mass spectrometer equipped with a glow discharge electron ionization source and a cylindrical ion trap mass analyzer has been employed for the detection, identification, and quantitation of gaseous samples of nine toxic industrial compounds (acrolein, acrylonitrile, carbon disulfide, cyanogen chloride, ethylene oxide, formaldehyde, hydrogen cyanide, phosgene, and sulfur dioxide). The apparatus was fitted with a combined direct air leak and dual thermal desorption tube inlet that facilitated continuous sampling of analytes with throughput times of 2 min or less. Most analytes exhibited a linear response over the concentration ranges studied (sub-parts per billion [ppb] to parts per million [ppm]). Sorbent tube limits of detection (20 ppb to 8 ppm for all analytes) were lower than those reported for the two compounds examined using direct leak (acrylonitrile 16 ppm and phosgene 500 ppb). All limits of detection were less than the concentration at which the analyte posed an imminent hazard to life and health. Sensitivity, probability of true positives, and the false positive rate for each compound were investigated and described by employing receiver operating characteristic (ROC) curves. Data of high quality with low false positive and negative rates suggest good chemical specificity and sensitivity of the apparatus. Complex matrices including of second-hand smoke, gasoline exhaust, diesel fuel exhaust, and multiple analytes were also investigated. Limits of detetcion for analytes generally increased in the mixtures. However, analytes could still detected at concentrations as low as 100 ppb.

17 Product Authenticity

Savaliya AA, Shah RP, Pradad B, Singh S*// *NIPER, Dept Pharmaceut Anal, Sector 67, IN-160062 Mohali, Punjab, India
J Pharm Biomed Anal 2010 **52** (3) 406
Screening of Indian aphrodisiac ayurvedic/herbal healthcare products for adulteration with sildenafil, tadalafil and/or vardenafil using LC/PDA and extracted ion LC-MS/TOF
Healthcare medicines including ayurvedic/herbal are ragarded as being safe in the belief that they are derived from natural products. However, there have been several recent reports worldwide regarding the adulteration of aphrodisiac herbal formulations with synthetic PDE-5 inhibitors. The present study was performed to explore the presence of synthetic PDE-5 inhibitors (sildenafil, tadalafil and/or vardenafil) in ayurvedic/herbal healthcare marketed in India for aphrodisiac/related uses. Eighty-five herbal formulations (HFs) were analyzed in the study. The concoctions were extracted with methanol and subjected to centrifugation. Early detection of the presence of sildenafil, tadalafil and vardenafil in the HFs was achieved through analysis of extracted ion mass chromatograms at the *m/z* values of respective parent ions, and two prominent fragments of each. To detect sildenafil and tadalafil adulteration was also detected by comparing the relative retention times (RR_T) and UV spectra. Further confirmation was accomplished by comparison of accurate mass spectra with those of the two available standards. Of the 85 HFs tested, only one was eventually found to be adulterated with sildenafil. The extent of adulterant in this sample was determined to be the therapeutic dose in the formulation. This research is suggestive of the emergence of adulteration of Indian herbal products with PDE-5 inhibitors.

18 Techniques

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Appl Radiat Isot 2010 **68** (6) 1012
Detection of drugs and explosives using neutron computerized tomography and artificial intelligence techniques
In respect of public security, a technique has been developed to detect illicit drugs and plastic explosives. A non-destructive assay with neutrons was employed and the procedure utilised real time neutron radiography together with computerized tomography. The protocol is based upon the application of an artificial intelligence technique and thus provided with automatic responses. Previously, using real samples, this approach proved capable of identifying 97% of the inspected materials

Jackson AU, Garcia-Reyes JF, Harper JD, Wiley JS, Molina-Diaz A, Ouyang Z, Cooks RG*// *Purdue Univ, Dept Chem, West Lafayette, In 47907, USA
Analyst 2010 **135** (5) 927
Analysis of drugs of abuse in biofluids by low temperature plasma (LTP) ionization mass spectrometry
Direct mass analysis of samples in their native atmospheric environment with little or no sample preparation is possible by the application of low temperature plasma (LTP) ionization. The low temperature plasma probe was employed in the direct and rapid analysis of aqueous phase samples including the biofluids saliva, urine, and hair extract by mass spectrometry. A detailed trace qualitative examination of 14 drugs of abuse has been carried out. Relative standard deviation was on average approximately 16% for the LTP analysis of the drugs of abuse standards. Eleven of the 14 drugs of abuse were detected in the low ng/ml (3 pg absolute detection) to the mid µg/ml (approximately 30 ng absolute detection) concentration range. However, one drug, namely cannabidiol, was not detectable until supplemental heating of the substrate was incorporated into the experimental procedure. Additional supplemental heating enhanced the detection limits by at least an order of magnitude to approximately 0.5 ng/ml to 0.5 µg/ml (1.5 pg-1.5 ng absolute) for 12 of the 14 drugs of abuse. This extended the linear dynamic range which for most analytes was four orders of magnitude. By employing a deuterated internal standard, the quantitative capabilities of the technique were evaluated in respect of benzoylecgonine in urine. Matrix effects noted during the analysis of the drugs in complex biological fluids are also discussed. Furthermore, low temperature plasma ionization was applied to the examination of real (but not spiked) biological samples. These results were confirmed using standard LC/MS methodology. A primary advantage of ambient desorption/ionization technique is the capability to directly analyse liquid surfaces thus facilitating *in situ* detection. In addition, the procedure exhibits remarkable sensitivity in the analysis of the drugs of abuse studied here. On-the-other-hand, a disadvantage is modest quantitative accuracy. Therefore, LTP is better as a rapid but semi-quantitative screening technique

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Anal Chem 2009 **81** (21) 8724
Directly heated high surface area solid phase microextraction sampler for rapid field forensic analyses
Dynamic sampling at high air velocities (up to several hundred centimeters per second) has been achieved by the development of a high-surface area solid phase microextraction (HSA-SPME) sampler. The sampling apparatus consists of a thin wire coated with carboxen/polydimethylsiloxane (carboxen/PDMS) material, wound in the annular space between two concentric glass tubes, providing a large trapping surface from which analytes may then be thermally desorbed with little power consumption upon resistive heating of the wire. Desorbed analytes are focused and reconcentrated on a microtrap. Subsequently this is resistively heated to introduce analytes for GC or GC/MS analysis. The efficiency of the HSA-SPME sampler was tested with benzene, toluene, ethylbenzene, and xylenes (BTEX) included in a 39-component toxic organics (TO-14) gas mixture. Quantitation of trace-level BTEX compounds present during weapons cleaning was completed using stepwise calibration. Detection limits of 0.2-6.9 $pptr_V$ were noted for these analytes using single ion monitoring GC/MS analysis. An improvement in sensitivity of several orders of magnitude was accomplished in comparison with standard dynamic flow SPME with a commercially available 10 mm carboxen/PDMS fiber. Rapid analyte uptake and improved sensitivity of the procedure employing the HSA-SPME design enables rapidl collection and analysis of VOC samples in field settings with a portable hand-held pump and a small, low power GC/MS instrument. This system will be particularly beneficial for situations involving forensics, public safety, and military defensive or intelligence needs where rapid, sensitive detection of airborne analytes is essential