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Current awareness in drug testing and analysis

1 Reviews

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Growth Horm IGF Res 2009 **19** (4) 399

Perspective: Proteomic approach to detect biomarkers of human growth hormone

A specific, sensitive, and reproducible kit for the detection of recombinant human growth hormone (rhGH) abuse has not been achieved despite the establishment of several serum biomarkers for rhGH when used alone or together. The search for further GH specific indicators continues. In this review, the focus is on the employment of proteomics in general and two-dimensional electrophoresis (2-DE) in particular for the discovery of new GH induced serum biomarkers. Also reviewed are some of the procedures involved in 2-DE. Finally, the potential of tissues other than blood for biomarker discovery is examined

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Growth Horm IGF Res 2009 **19** (4) 388

Surface plasmon resonance immunoassays - A perspective

An extremely challenging task from an anti-doping viewpoint is the detection of human growth hormone (GH) misuse. GH is an endogenous hormone existing at very low levels in the circulation (for the most abundant 22kDa isoform approximately 50pM in plasma and 100fM in urine) either as monomer or homo- and heterodimers. It includes a collection of distinct isoforms. It undergoes pulsatile secretion that may affected by many different endogenous and exogenous factors. However, following administration of the recombinant, single-isoform pharmaceutical, the feedback to the pituitary results in a reduction of the endogenous heterogeneous hormone causing altered ratios between the different GH isoforms. Therefore, measurement of the isoform ratios with immunoassays appears to be the method of choice. Conventional assays do not produce information on isoform-specific association and dissociation events of the individual primary antibody-isoform or isoform-secondary antibody interactions. These particular data may be produced by employing surface plasmon resonance (SPR) which facilitates monitoring of biomolecular interactions in a dynamic and label-free setting. The different aspects of SPR are described. Also, how the technology may be beneficial for understanding today's anti-GH immunoassays. Finally, whether this procedure might be utilised for measurement of GH in the near future

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Growth Horm IGF Res 2009 **19** (4) 346

Moving one step closer to catching the GH cheats: The GH-2004 experience

Growth hormone has both anabolic and lipolytic properties. Consequently, it is abused by athletes to promote performance. The detection of GH abuse presents challenges; it is an endogenous hormone which varies widely in any one day in terms of concentration. The GH-2000 project proposed a protocol employing the measurement of IGF-I and type III pro-collagen (P-III-P). However, when the results of the GH-2000 project were presented to an expert workshop, the technique was supported but it was suggested that several issues required to be resolved before the procedure could be adopted. The first issue related to ethnicity as most subjects in the GH-2000 were Caucasians. Secondly, there was the confounding effect of injury because P-III-P is a marker of soft tissue turnover. Consequently, the GH-2004 project was devised to address these issues. The GH-2004 project demonstrated that whereas there are minor differences in IGF-I and P-III-P between ethnicities, they are small and do not influence the performance of the test. Injury results in a small increase in P-III-P but once more this is of insufficient magnitude to be of concern to the test performance. The GH-2004 project has produced further endorsement for the marker approach as a technique for detecting GH abuse in athletes. WADA have not developed their own immunoassays. Further work is required to authenticate newer commercial assays measuring IGF-I and P-III-P in order to establish reliable conversion factors to the original GH-2000 units which will allow the published formulae to be utilised

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Forensic Sci Int 2009 **189** (1-3) 1

An assessment of sensing technologies for the detection of clandestine methamphetamine drug laboratories

One of the most significant social challenges facing most societies are clandestine drug laboratories involved in the production of illicit drugs. In North America, clandestine methamphetamine production is especially important and is associated with notable effects on health, safety, and the environment. Since many of these production facilities are temporary and capable of producing large quantities of prohibited drugs in cycles that may often occupy less than 48 h, timely discovery essential. This paper assesses sensing technologies capable of detecting the effluents commonly released during the production cycle for the various methods. A brief review of the most common

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.

methamphetamine manufacturing processes is included and target gases are identified. Each methamphetamine manufacturing process has a unique temporal chemical signature. Therefore, it is possible that this profile may be employed to identify an illicit methamphetamine laboratory from legitimate sources of these gases. In respect of the target gases, the paper provides an assessment of both commercial and research stage sensor technology. The results of these comparisons are utilised to make conclusions about the most suitable sensing technologies for illegal methamphetamine laboratory detection

2 Sports Doping - General

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J Anal Chem Engl Tr 2009 **64** (8) 832

Simultaneous determination of a broad spectrum of nonconjugated xenobiotics by high-performance liquid chromatography-tandem mass spectrometry

A protocol was developed for screening of 23 corticosteroids, 12 anabolic steroids, 23 diuretics, and 49 central nervous system stimulants and narcotics. Sample preparation incorporates extraction, evaporation of the organic extract in a nitrogen flow, and dissolution of the dry residue. The reconstituted samples were analyzed by high-performance liquid chromatography-tandem mass spectrometry with atmospheric pressure electrospray ionization under different recording conditions. Negatively charged ions were recorded to determine corticosteroids and diuretics. Positively charged ions were employed for anabolic steroids, diuretics, stimulants, and narcotics. Mass spectra were produced for all test compounds. Characteristic ions, retention times, detection limits, degree of ionization suppression by the matrix, and recovery were obtained for all analytes

3 Steroids

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Int J Mass Spectrom 2009 **285** (1-2) 58

Statistical analysis of fragmentation patterns of electron ionization mass spectra of enolized- trimethylsilylated anabolic androgenic steroids

Included in the list of prohibited substances of the World Anti-Doping Agency (WADA) as substances abused to enhance athletic performance are anabolic androgenic steroids (AAS). Gas chromatography coupled to mass spectrometry (GC–MS) plays an significant role in doping control analyses by identifying AAS as their enolized-trimethylsilyl (TMS)-derivatives using the electron ionization (EI) mode. The aim of this study was to examine the suitability of complementary GC–MS mass spectra and statistical analysis (principal component analysis, PCA and partial least squares-discriminant analysis, PLS-DA) to differentiate AAS in respect of their structural and conformational features expressed by their fragment ions. PCA resulted in a classification among the AAS molecules which became more evident following application of PLS-DA to the dataset. PLS-DA produced a clear distinction of the AAS molecules which included 1-ene-3-keto, 3-hydroxyl with saturated A-ring, 1-ene-3-hydroxyl, 4-ene-3-keto, 1,4-diene-3-keto and 3-keto with saturated A-ring anabolic steroids. In addition, structurally diagnostic fragment ions and dissociation routes are revealed providing data for the determination of unknown AAS or chemically modified molecules known as designer steroids

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Biosens Bioelectron 2009 **24** (12) 3562

A sensitive voltammetric sensor for determination of synthetic corticosteroid triamcinolone, abused for doping

Triamcinolone is sometimes abused by athletes for doping prposes. Edge plane pyrolytic graphite electrode (EPPGE) modified with single-wall carbon nanotubes (SWNTs) has been used to develop a sensor. When compared with the voltammetric behavior between SWNTs modified EPPGE and fullerene - C_{60}-modified EPPGE showed that SWNTs modified EPPGE to be more sensitive. The electrode demonstrated an effective catalytic response with good reproducibility and stability. The influence of several factors such as pH, square wave frequency and steroid concentration were studied. The square wave voltammetric response of the electrode to triamcinolone is linear in the range 0.1-25 nM with a detection limit and sensitivity of 8.9 x 10^{-10}M and 2.06 μA/nM, respectively. The sensor was employed for the analysis of triamcinolone in several commercially available pharmaceuticals and real urine samples obtained from patients undergoing pharmacological treatment with triamcinolone. When the results were compared with HPLC analysis, there was satisfactory agreement. The product obtained after reduction of triamcinolone was also characterized using ^{1}H NMR and GC-MS. The site of reduction was found to be carbonyl group at position 20. The sensor is rapid, simple and accurate and can be readily utilised for detecting cases of doping

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Steroids 2009 **74** (10-11) 837

Detection and structural investigation of metabolites of stanozolol in human urine by liquid chromatography tandem mass spectrometry

The utility of LC-MS/MS in precursor ion scan mode for the determinaton of urinary stanozolol metabolites has been investigated. The product ion at *m/z* 81 has been selected as specific for stanozolol metabolites without a modification in A- or N-rings and the product ions at *m/z* 97 and 145 for the metabolites hydroxylated in the N-ring and 4-hydroxy-stanozolol metabolites, respectively. By employing this protocol, the parent drug and up to 15 metabolites were identified in a positive doping test sample. In addition, the study of a specimen from a chimeric uPA-SCID mouse collected after the administration of stanozolol revealed the presence of 4 other metabolites. The data obtained from the product ion spectra was emplyed to develop a SRM method for the detection of all 19 compounds. This SRM procedure was employed with several doping positive samples. All metabolites were identified both in the uPA-SCID mouse sample and positive human samples and were not detected in none of the blank samples tested. The metabolic nature of all the detected compounds was verified. Furthermore, the application of the SRM technique to a single human excretion study revealed that one of the metabolites (4ξ,16ξ-dihydroxy-stanozolol) was detectable in negative ionization mode for a longer period than those commonly utilised in the screening of stanozolol misuse (3'-hydroxy-stanozolol, 16β-hydroxy-stanozolol and 4β-hydroxy-stanozolol) in doping analysis. The utilisation of the described protocol to several positive doping samples verified the applicability of this metabolite for the screening of stanozolol misuse. A tentative structure for each of the metabolites is presented based on the product ion spectra measured with accurate masses using UPLC-QTOF MS

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J Chromatogr B 2009 **877** (23) 2321

Validation and performance comparison of two carbon isotope ratio methods to control the misuse of androgens in humans

Carbon isotope ratio of androgens is routinely performed in urinalysis to distinguish the abuse of testosterone or testosterone prohormones by athletes. Recently, increasing application of gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) for target and systematic investigations of samples has necessitated development of rapid sample throughput as well as high selectivity in the extraction process particularly in the case of conspicuous samples. Consequently, the complimentary use of an SPE-based assay and an HPLC fractionation method as a two-stage strategy for the isolation of testosterone metabolites and endogenous reference compounds prior to GC/C/IRMS analyses has been examined. Assays evaluation demonstrated acceptable performance in terms of intermediate precision (range: 0.1-0.4 per thousand). In addition, Bland-Altman analyses revealed no significant bias (0.2 per thousand). To further validate this two-stage procedure, all the specimens (n = 124) collected during a major sport event were analysed

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J Food Sci 2009 **74** (8) T67

Development of a solid-phase extraction-enzyme-linked immunosorbent assay for the determination of 17 -19-nortestosterone levels in antifatigue functional foods

17β-19-Nortestosterone (17β-NT) has been illegally used in antifatigue functional foods to promote muscle growth and improve endurance. A rapid and sensitive solid-phase extraction-enzyme-linked immunosorbent assay (SPE-ELISA) method was developed and successfully applied to analyze the levels of 17β-NT in antifatigue functional foods. A polyclonal antibody against 17β-NT was produced from rabbits immunized with the 17β-NT-BSA conjugate, and a competitive direct enzyme-linked immunosorbent assay was developed for the rapid detection of 17β-NT. The concentration causing 50% inhibition (IC_{50}) and the limit of detection (LOD) were found to be 0.08 and 0.0055 ng/ml, respectively; this was better than methods previously reported that had

a LOD of 2.4 ng/ml. C_{18} cartridges were investigated for use in removing the effects of matrix in foods, and the sample purification protocol was optimized. Using the developed SPE-ELISA method, recoveries of functional food samples were obtained in the range of 71% to 91.5%. Moreover, 2 kinds of antifatigue functional foods were analyzed using the established ELISA and HPLC methods. The correlation coefficient of the results obtained using the 2 methods was greater than 0.98. Thus, the preliminary evaluation of the SPE-ELISA method proved that it is a specific, sensitive, and precise tool that can be used for the practical detection of 17β-NT in various antifatigue functional food samples

4 Peptides

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Growth Horm IGF Res 2009 **19** (4) 369
Detection of GH abuse in sport: Past, present and future
Human growth hormone (hGH) is abused as a doping agent in sport because of its considered performance enhancing effects. However, its misuse also poses potentially serious adverse effects to a person's health. Therefore, hGH and its releasing factors are prohibited in sport. Thus, it has been included in the Prohibited List which is updated and published yearly by the World Anti-Doping Agency (WADA). The sports movements and the anti-doping authorities, originally led by the International Olympic Committee (IOC) and later by WADA, have made substantial efforts into developing methods for its detection. Such research is intende to mitigate the danger that hGH doping poses to the spirit of sport and to the health of athletes. Presently, a primary analytical method, the isoform differential immunoassay, has been employed in WADA-accredited laboratories. Concurrently, a second, indirect protocol for the detection of hGH misuse, based on the quantification of hGH-associated biological indicators, has been produced. Ultimately, the aim is to combine both protocols to increase the sensitivity and expand the period for detection of doping with hGH. Furthermore, new analytical methods utilising proteomic and genomic technologies as well as the use of mass spectrometry-based techniques of detection are being examined for future deployment in hGH anti-doping tests

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Growth Horm IGF Res 2009 **19** (4) 361
Statistical issues in implementing the marker method
In addition to biochemical issues, statistical problems arise during the detection of growth hormone (GH) abuse by athletes. Statistical approaches to the various issues which have arisen during the work of the GH-2000 and GH-2004 teams are outlined. The requirement to develop a test which detects GH abuse in any elite athlete 'beyond reasonable doubt' is particularly important. While minimising the risk of false accusation, the protocol should be robust enough to withstand any legal challenge. Various concerns which arise in the development of such a test are identified. In addition, how these issues were resolved are described. The abuse of GH cannot be detected directly because it is an endogenous hormone whose concentration varies substantially. The procedure under consideration here employs markers whose levels are more stable but are influenced by GH. The statistical techniques utilised are designed to optimise use of these markers whilst accounting for all factors contributing to errors in measurement. There were two salient stages in the statistical investigation conducted to develop the GH detection algorithm. Firstly, was the necessity to establish GH-dependent biomarkers which would identify GH doping reliably and robustly for a significant period. Secondly, was the requirement to calibrate the GH detection procedure in the elite athlete population. Consequently, the technique should be applicable to all athletes, irrespective of age, sex and ethnicity and regardless of whether they had recently sustained an injury. In practice, additional studies were required to ensure that the protcol met the WADA testing standards. In addition, the proposed method might be employed by any WADA accredited laboratory without placing any athlete at an unfair disadvantage and ensuring a high level of confidence in any result obtained

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Growth Horm IGF Res 2009 **19** (4) 383
Detection of growth hormone responsive proteins using SELDI-TOF mass spectrometry
A significant problem in elite sports is the detection of growth hormone (GH) doping. GH is an endogenous hormone and secreted in a pulsatile pattern from the anterior pituitary. This may be affected by a variety of physiological and pathophysiological conditions. Recombinant hGH is hardly distinguishable from the predominant endogenous isoform and is cleared from the body within 24h. GH is included on the World Anti-Doping Agency list of banned substances. However, the detection of GH misuse remains problematic. An overview is provided of the potential application of surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry to examine proteomic changes following GH administration employing both serum and white blood cell extracts as samples for analysis. To date, indications are that proteomic changes observed following GH administration have the possibility to produce novel indicator sets for the detection of GH abuse

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Growth Horm IGF Res 2009 **19** (4) 357
Laboratory issues in the implementation of the marker method
IGF-I and P-III-P are two notable indicators in the identification of growth hormone or IGF-I abuse. These compounds may be determined in plasma or preferably in serum. There are several commercial assays for IGF-I but only two for P-III-P. The principles on which these assays are based and the choice of appropriate examples for doping control purposes are discussed. Future prospects for quantification using mass spectrometry are also briefly discussed

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Growth Horm IGF Res 2009 **19** (4) 408
IGF-I abuse in sport: Current knowledge and future prospects for detection
As the tests for detecting growth hormone (GH) abuse progress and improve, it is probable that athletes will abuse insulin-like growth factor-I (IGF-I) because IGF-I mediates many of the anabolic actions of growth hormone. IGF-I promotes muscle protein synthesis, glycogen storage and lipolysis. These effects make IGF-I desirable as a potential performance-enhancing agent. Pharmaceutical companies are producing commercial preparations of recombinant human IGF-I (rhIGF-I) to treat disorders of growth. The opportunity for athletes to acquire supplies of rhIGF-I on the black market has followed increased legal availability. Whereas the long-term effects of IGF-I administration have to be established, it is likely that they will be similar to the adverse effects of chronic GH abuse. Therefore, the identification of IGF-I misuse is a challenge for the anti-doping organisations. Research has already started into the production of a test for IGF-I abuse employing markers of GH action. Concurrently, the effects of rhIGF-I on physical fitness, body composition and substrate utilisation in healthy volunteers are being studied

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Growth Horm IGF Res 2009 **19** (4) 352
20K-GH and its use in detecting GH abuse
Several studies have investigated the physiology of a GH isoform (20K-GH) following its successful production with recombinant DNA technology. This report analyses studies of its biological effect, measurement and secretion. In order to employ serum 20K-GH level for detecting GH abuse, a new procedure was developed. Serum 22K-GH, 20K-GH were measured in normal subjects and athletes but no abnormal results were found among the latter. Another study established that following the exogenous administration of 22K-GH, serum 22K-GH increased remarkably and 20K-GH decreased. The interval was relatively short, lasting approximately 24-36h in our and other studies. An increasing ratio, 22K-GH:20K-GH was the most appropriate biomarker of GH abuse. Studies supported by the WADA were conducted in collaboration with an Australian group. A new protocol for assay of the GH isoform by employing a beads assay platform is being devised. The detection of GH abuse by direct measurement of 20K-GH is a valid scientific approach. However, the duration of the positive results is short. The procedure in combination with the marker method will facilitate out-of-competition testing or investigation of target cases. In addition, its employment in the doping test passport is considered to be a possible future strategy

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Am J Hematol 2009 **84** (11) 755
Modification of gene expression: Help to detect doping with erythropoiesis-stimulating agents (Letter)
No abstract available

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Growth Horm IGF Res 2009 **19** (4) 366
Overview of the legal framework applicable to the marker method for the detection of human growth hormone
There is an ongoing race in the world of doping and anti-doping in sport

between athletes who try to gain an unfair advantage through the use of prohibited substances or methods and those who want to keep the sport free of drug misuse. Sophisticated drug misuse frequently involves compounds that are difficult to detect. This presents both scientific and legal challenges for implementing new detection protocols. An overview is presented of the legal considerations in sports drug testing that may apply to the use of the marker method for the identification of human growth hormone. Where the blood markers IGF-1 and P-III-P suggest misuse of exogenous growth hormone, reference to positivity criteria and laboratory uncertainty measurements would aid anti-doping adjudicative bodies in establishing, to their reasonable confidence, that a positive test has been performed. Employment of established positivity criteria and laboratory uncertainty measurements position of the marker method for growth hormone within the existing, accepted legal framework for interpreting an adverse analytical finding

5 Diuretics

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J Chromatogr A 2009 **1216** (31) 5819

Qualitative detection of diuretics and acidic metabolites of other doping agents in human urine by high-performance liquid chromatography-tandem mass spectrometry: Comparison between liquid-liquid extraction and direct injection

Urinalysis employing direct injection of urine has incresed in interest in the field of analytical toxicology, including doping control analysis. Unfortunately, direct urinalysis by a LC-MS/MS method for 34 diuretics and 9 other doping agents produced several analytical problems not observed using a ususal liquid-liquid extraction. Consequently, a comparison was made between liquid-liquid extraction and direct injection. Validation results indicated that the liquid-liquid extraction at pH 7 facilitates analysis without major drawbacks regarding matrix effects. Good sensitivity was noted and detection limits ranged between 1 and 250 ng/ml for all compounds. However, with the direct injection approach shifted retention times were observed for several acidic and basic compounds due to interfering matrix effects. This could be reduced by a 25-fold dilution of the urine samples. In addition to the improved retention time stability, the diluted samples also showed lower ion suppression than the undiluted ones. Following a 25-fold dilution, detection limits ranged between 10 and 250 ng/ml for all compounds. The detection limits are at or below the minimum required performance level required by the World Anti-Doping Agency and thus this method could be applied to routine anti-doping analysis. Previously declared positive samples were reinvestigated using both the liquid-liquid extraction and direct injection. With both techniques all 26 samples were found to be positive, showing the applicability of direct injection for the analysis of diuretics

7 Equine

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Anal Bioanal Chem 2009 **394** (8) 2119

Development of a metabonomic approach based on LC-ESI-HRMS measurements for profiling of metabolic changes induced by recombinant equine growth hormone in horse urine

Recombinant equine growth hormone (reGH), a growth promoter, is suspected to be used in horseracing to improve performances in spite of a worldwide existing regulation banning its use. Various analytical techniques previously described to screen for its misuse have experienced some shortcomings in terms of detection period. This especially applies during the first days after reGH administration. In order to produce a novel screening tool for growth hormone abuse in horseracing, a strategy involving the characterization of global metabolomic fingerprints in urine samples of non-treated and reGH-treated horses by liquid chromatography-electrospray-high-resolution mass spectrometry (LC-ESI-HRMS) has been developed and evaluated. This procedure includes limited sample preparation of the samples and the use of appropriate software for data processing and analysis. Preliminary work assessed the reproducibility of both sample preparation and mass spectrometry (MS) measurements in order to demonstrate the reliability of the technique. The developed protocol was employed with two horses and illustrated the applicability of the procedure. Preliminary results demonstrated significant modifications of the metabolome after treatment with reGH

8 Recreational Drugs - General

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Clin Chem Lab Med 2009 **47** (7) 854

Therapeutic drug monitoring and drugs of abuse testing on the cobas 6000 analyzer series: Analytical performance under routine-like conditions

By employing a spectrum of representative assays, the analytical performance of the clinical chemistry module c 501 (cobas 6000 analyzer series) was determined both for therapeutic drug monitoring and drugs of abuse testing. Using a simulated routine workload, attention was especially paid to potential interactions between reagents. Within-run and total imprecision were analysed with a selection of representative reagents. Deviation from a consensus mean was determined by employing samples from a proficiency testing scheme. Using routine samples, method comparison was performed out against the MODULAR ANALYTICS SWA and COBAS INTEGRA 800 analysis systems. Total coefficients of variation (CV) ranged from 1.9% to 7.8% for individual drugs, and from 3.2% to 8.6% for drugs of abuse testing. Results from proficiency test samples were between 81% and 125% of the consensus mean for therapeutic drugs. Method comparisons (Passing-Bablok regression) showed appropriate performance to MODULAR ANALYTICS SWA and COBAS INTEGRA 800 systems, with slopes from 0.93 to 1.17 and correlation coefficients r > 0.98. Imprecision in a simulated routine run was examined using a total of 42 methods (10 therapeutic drug monitoring, 9 drugs of abuse testing, 3 enzymes, 12 substrates, 8 specific protein assays). Reference batch run imprecision ranged from 0.7% to 5.0% CV for therapeutic drug monitoring assays, except for digoxin (DIG) (7.3%), and from 0.9% to 7.7% for drugs of abuse testing. The CVs of general clinical chemistry and specific protein tests were within the expected limits of 2% and 4%. CV changes in the simulated routine run were within the expected limits for most assays. Negative DeltaCVs (> or = 2%) for DIG, digitoxin (DIGIT), cannabinoids (THC), and phencyclidine (PCP) may indicate improved performance when running these assays in a simulated routine operation. A positive DeltaCV (> or = 3%) was found for amphetamines (AMPHs). The cobas c 501 module appears applicable for routine use as consolidated workstation. With the exception of a potential interaction with AMPH, as indicated by the positive DeltaCV, no significant interferences from different reagents were noted

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Electrophoresis 2009 **30** (14) 2583

Analysis of multiple abused drugs and hypnotics in urine by sweeping CE

Multiple drugs usage is very common in addicts (AD). However, some parent drugs were undetectable in urine, it was necessary to monitor their metabolites simultaneously. A sweeping CE was established for the determination of several kinds of abused drug and their metabolites in AD urine. This method was developed using chemometric experimental design to simplify the CE optimization. The capillary was filled by separation buffer (phosphate buffer (75 mM, pH 2.5) and methanol (70:30 v/v)) and then hydrodynamically injected large volume of samples into capillary (1 psi, 200 s). Following was using sweeping buffer (phosphate buffer (75 mM, pH 2.5) and methanol (90:10 v/v) containing 65 mM SDS) to sweep and stack analytes. The separation voltage was set at -15 kV (anode at detector end). During method validation, calibration plots were linear (r > or = 0.992) over a range of 0.1-3 μg/ml for codeine, ketamine, and methamphetamine, 0.15-3 μg/ml for morphine, 0.1-1 μg/ml for alprazolam and oxazepam, and 0.1-1.2 μg/ml for other other benzodiazepines and its metabolites. During intra- and inter-day analysis, relative standards and relative errors were less than 14%. The analytes could be simultaneously analyzed and have a detection limit down to 20-50 ng/ml (S/N = 3). The results showed good coincidence with GC-MS or LC-ESI-MS. This method was feasible for application to detect trace levels of abused drugs in AD' urine

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J Anal Toxicol 2009 **33** (7) 356

Comprehensive analysis of drugs of abuse in urine using disposable pipette extraction

A disposable pipette extraction technique (DPX) has been developed for the isolation of basic, acidic, and neutral drugs of abuse from a low volume of urine (0.2 ml). DPX is a solid-phase extraction device that uses loosely contained sorbent inside a pipette tip fitted with a screen. Conditioning steps are not required. Therefore, faster extraction times are achieved. The DPX protocol employed here utilises a modified divinyl benzene sorbent containing both cation-exchange and reversed-phase mechanisms that facilitates the retention

of basic and acidic/neutral drugs, respectively. Comprehensive urinanalysis was accomplished for a diverse group of drugs and drug classes in urine including amphetamines, opiates, cocaine and its metabolites, tetrahydrocannabinol metabolite, tricyclic antidepressants, meperidine, methadone, and phencyclidine. Recoveries of most drugs of abuse were 90% or greater with relative standard deviations of less than 10%. Further validation included the analysis of urine specimens previously analyzed by a local forensic toxicology laboratory

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J Chromatogr A 2009 **1216** (38) 6587

Application of Snyder-Dolan classification scheme to the selection of "orthogonal" columns for fast screening of illicit drugs and impurity profiling of pharmaceuticals - I. Isocratic elution

Fourteen carefully selected reversed phase columns were employed with 18 cationic drug solutes under the isocratic elution conditions advised in the Snyder-Dolan (S-D) hydrophobic subtraction procedure of column classification. The standard errors (S.E.) of the least squares regressions of log *k' vs*. log k'_{REF} were produced for a specific column against a reference column and employed to compare and classify them with respect to their selectivity. The data are equable with those produced in an examination of 16 test solutes recommended by Snyder and Dolan. In respect to the extent that the drugs are representative, the data show that the S-D classification scheme is also generally relevant to pharmaceuticals under isocratic conditions. Thus, those columns determined to be similar based on the 16 S-D solutes were also analagous based on the 18 drugs. In addition, those columns estimated to have significantly different selectivities based on the 16 S-D probes also appeared to be quite different for the drugs. Since the S-D protocol has been employed to classify more than 400 different types of reversed phases, the expansion to cationic drugs is a significant finding

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J Chromatogr B 2009 **877** (18-19) 1743

Optimization of a rapid microwave-assisted extraction method for the simultaneous determination of opiates, cocaine and their metabolites in human hair

Six illegal drugs of abuse (cocaine, benzoylecgonine (BZE), cocaethylene (CCE), morphine, 6-monoacethylmorphine (6AM) and codeine) in human hair samples were simultaneously investigated with a rapid and cleanup-free microwave-assisted extraction (MAE) method. Analytes were determined using high performance liquid chromatography (HPLC) with photodiode array UV detection. The effects of several parameters on the efficiency of the MAE procedure were investigated in detail by a multi-objective optimization approach based on a hybrid experimental design (17 experiments) and desirability functions. Six drugs were successfully extracted from human hair with recoveries close to 100% and good reproducibility (<3.6% RSD) under the optimal MAE conditions: 11 ml dichloromethane (DCM) extraction solvent, 60ºC extraction temperature, 9 min extraction time and 0.5 ml of methanol (MeOH) added to 50mg of the hair sample in the extraction vessels. Limits of quantification of 0.2 ng/mg were found for the above compounds. Sample preparation procedures, including MAE, enzymatic digestion and digestion by aqueous acids were compared. Results suggest that the global behaviour of sample procedures provided similar satisfactory recoveries ranging from 86 to 100%. Importantly, the MAE procedure facilitated a reduction of extraction time by 100-fold and the elimination of cleanup steps. Slightly higher recoveries of morphine, 6AM, BZE and CCE, at 1 ng/mg concentration level and cocaine at 40 ng/mg concentration level, were achieved using MAE. Finally, the proposed MAE method was employed to analyse several human hair samples from multidrug abusers

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Forensic Sci Int 2009 **189** (1-3) 88

Cocaine and heroin in waste water plants: A 1-year study in the city of Florence, Italy

The spread and trends in use of a drug of abuse is important in policy planning of strategies aiming at deterrence or the fight against drug trafficking. The spread of illicit drugs in a population is difficult to assess but among the various measures available, the analysis of waste water plants represents one of the most reliable source of data. Waste water was analysed in order to monitor illicit drug use by a local population. Specifically, cocaine and heroin were investigated in the city of Florence, Italy, over a 1-year (July 2006-June 2007) period using state-of-the-art measuring techniques from waste water samples. Cocaine, benzoylecgonine, and morphine were analysed from water samples by gas chromatography-mass spectrometer, and the amount of illicit substance was determined. Data suggest a bimodal distribution for cocaine (December and March), whereas heroin showed a main peak in April. The heroin-to-cocaine use ratio in terms of estimated doses per month ranged from 0.11 to 0.76, indicating wider distribution of cocaine than heroin in Florence. Waste water analysis may become a valuable tool in monitoring use of illicit drugs over time. It is especially useful to highlight changes in the magnitude and relative use of illicit drug at a population level thereby facilitating the development strategies against drug trafficking and abuse. If routinely performed, it could be part of epidemiologic surveillance programmes on drug abuse

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Analyst 2009 **134** (9) 1834

Disposable microfluidic device with ultraviolet detection for highly resolved screening of illicit drugs

By conveniently integrating one poly(methyl methacrylate) board with four reservoirs and one fractured fused-silica capillary with 50 μm i.d. and 7.5 cm total length on a printed circuit board for applying sampling and separation voltages, disposable microfluidic device was fabricated. When combined with a home-made ultraviolet workstation it could be conveniently employed for efficient screening and quantitative detection of μg/ml illicit drugs. With eight illicit drugs as models, they were baseline-separated within 240 s with the separation efficiency up to 600,047 plates/m at the designed device. The developed device and proposed protocol were successfully employed to screen illicit drugs in human urine. The paper describes a simple and low-cost procedure to construct the microfluidic device and provides a powerful way for sensitive and specific multi-screening of different drugs with high resolution, fast separation and low-cost

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Anal Bioanal Chem 2009 **395** (3) 819

Analysis of drugs of abuse in wastewater by hydrophilic interaction liquid chromatography-tandem mass spectrometry

Nine drugs of abuse (DOAs) and their metabolites (amphetamine, methamphetamine, methylenedioxymethamphetamine, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, cocaine, benzoylecgonine, ecgonine methyl ester and 6-monoacetylmorphine) were simultaneously analysed in wastewater using hydrophilic interaction liquid chromatography (HILIC) coupled to tandem mass spectrometry (MS/MS). The procedure was optimised and validated. The deuterated analogue was used for quantificationof each analyte. Separation emplying HILIC exhibited good performance for all drugs, particularly the hydrophilic compounds which elute early (amphetamine-like stimulants) or show no retention (ecgonine methyl ester) in reversed-phase liquid chromatography. Sample preparation utilising solid-phase extraction was optimised by comparing Oasis HLB and Oasis MCX sorbents for various parameters such as sample pH, amount of sorbent bed and washing solvent. The technique was authenticated for each compound in respect of the following parameters (following International Conference on Harmonisation guidelines): specificity, limit of quantification (LOQ), linearity, accuracy, precision, recovery and matrix effects. LOQs were 2 ng/l for 6-monoacetylmorphine, ecgonine methyl ester and amphetamine and 1 ng/l for the rest of the compounds, corresponding with the lowest point in the calibration curve. With the exception of 6-monoacetylmorphine, all drugs were detected from 1 to 819 ng/l in influent wastewater samples (n = 12) collected from 11 different wastewater treatment plants across Belgium. The presence of ecgonine methyl ester in wastewaterwas demonstrated for the first time. Prospective work will apply the new HILIC-MS/MS protocol in the assessment of the use of DOAs in Belgium by means of the "sewage epidemiology" approach

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Forensic Sci Int 2009 **189** (1-3) 100

The spectroscopic detection of drugs of abuse on textile fibres after recovery with adhesive lifters

Fibres are one of the most common forms of evidence associated with forensic investigations. Adhesive lifters to recover fibres from crime scene samples are a long established technique as an effective method to recover such items of evidence. After the fibres have been lifted they are placed in evidence bags for storage, safe transport and to maintain the chain of evidence. This investigation demonstrates that when fibres lifted with adhesive tape, particles of trapped substances are also lifted. Fibres of cotton, linen and wool were examined in colours ranging from white to black. Samples of seized Ecstasy, cocaine, ketamine and amphetamine were provided by East Sussex Police and by the

TICTAC unit at St Georges Hospital Tooting. Raman spectra of the fibres demonstrated that it is possible to identify drugs of abuse from trapped particles without interference from the fibres themselves. In addition, it was possible to obtain spectra without the detection process being compromised. Furthermore, Raman spectra from particles of drugs of abuse within fibres following tape lifting could be recorded through evidence bags. Once more, the detection process was not compromised. This initial study has demonstrated that fibres have the potential to provide greater levels of evidence than they do currently. Individuals that have had drugs of abuse about their person may have trace amounts of these substances trapped within the fibres of their clothing. This may be of particular importance if for example, drugs have been carried in pockets of a garment. Raman spectroscopy has the advantage of being non-destructive, allows for re-testing, requires no sample preparation and also can be performed on samples without removal from the evidence bag thereby removing any potential risk of contamination. However, it is necessary to consider that when dealing with trace amounts of drugs of abuse, the presence of such particles may be due to innocent transfer. The detection of particles of drugs of abuse on clothing does not necessarily provide proof of criminal activity in the absence of other evidence

9 Stimulants

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Addiction 2009 **104** (11) 1874
The spatial epidemiology of cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) use: A demonstration using a population measure of community drug load derived from municipal wastewater
The aim of this study was to determine the utility of community-wide drug testing with wastewater samples as a population measure of community drug use and to test the hypothesis that the association with urbanicity would vary for three different stimulant drugs of abuse. Single-day samples were obtained from a convenience sample of 96 municipalities representing 65% of the population of the State of Oregon. Chemical analysis of 24-hour composite influent samples for benzoylecgonine (BZE, a cocaine metabolite), methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA). The distribution of community index drug loads accounting for total wastewater flow (i.e. dilution) and population are reported. The distribution of wastewater-derived drug index loads was found to correspond with expected epidemiological drug patterns. Index loads of BZE were significantly higher in urban areas and below detection in many rural areas. Conversely, methamphetamine was present in all municipalities, with no significant differences in index loads by urbanicity. MDMA was at quantifiable levels in fewer than half the communities, with a significant trend towards higher index loads in more urban areas. This demonstration provides the first evidence of the utility of wastewater-derived community drug loads for spatial analyses. Such data have the potential to improve dramatically the measurement of the true level and distribution of a range of drugs. Drug index load data provide information for all people in a community and are potentially applicable to a much larger proportion of the total population than existing measures

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Electrophoresis 2009 **30** (12) 2238
Simple method for determination of cocaine and main metabolites in urine by CE coupled to MS
By employing CE coupled to MS *via* electrospray ionization (CE-ESI-MS) a simple method for the simultaneous determination of cocaine (COC) and five COC metabolites (benzoylecgonine, cocaethylene (CET), anhydroecgonine, anhydroecgonine methyl ester and ecgonine methyl ester) in human urine was developed and validated. Formic acid at 1 mol/l concentration was used as electrolyte whereas formic acid at 0.05 mol/l concentration in 1:1 methanol:water composed the coaxial sheath liquid at the ESI nozzle. The developed method presented good linearity in the dynamic range from 250 ng/ml to 5000 ng/ml (coefficient of determination greater than 0.98 for all compounds). LODs (signal-to-noise ratio of 3) were 100 ng/ml for COC and CET and 250 ng/ml for the other studied metabolites whereas LOQ's (signal-to-noise ratio of 10) were 250 ng/ml for COC and CET and 500 ng/ml for all other compounds. Intra-day precision and recovery tests estimated at three different concentration levels (500, 1500 and 5000 ng/ml) provided RSD lower than 10% (except anhydroecgonine, 18% RSD) and recoveries from 83-109% for all analytes. The method was successfully applied to real cases. For the positive urine samples, the presence of COC and its metabolites was further confirmed by MS/MS experiments

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Forensic Sci Int 2009 **189** (1-3) 24
Pharmacokinetics of disappearance of cocaine from hair after discontinuation of drug use
Monitoring of compliance with drug abstinence is assisted by methods that employ detection of drugs of abuse in hair. Therefore, it is important to understand the metabolism and rate of disappearance of drugs from hair in both clinical and forensic settings. The present study was designed to evaluate the kinetics of disappearance of cocaine and its metabolite, benzoylecgonine (BE), from hair following discontinuation of drug use. The Motherisk laboratory at the Hospital for Sick Children in Toronto routinely receives hair samples for toxicology analysis. Cocaine and BE hair results were derived from the Motherisk Database for calculation of half-life of these compounds in hair. Subjects were included in the study if they had gradually decreasing concentrations of cocaine and/or BE in sequential hair samples, with higher levels in the 1-3 cm distal segments (i.e. earlier in time) and low or non-measurable levels in the segment closest to the scalp (i.e. closer to the date of sampling). Elimination half-life of cocaine and BE in hair was derived *via* standard kinetics calculations. The study was anonymous, and received ethics approval by the Ethics Review Board of our institution. One hundred and thirty seven subjects met the inclusion criteria for the study. The median half-life of cocaine in hair was 1.5 months (95% CI 1.2-1.8) in females and 1.5 months (95% CI 1.1-1.8) in males. The median half-life of BE was 1.5 months (95% CI 1.1-2) in females and 1.5 months (95% CI 0.8-1.8) in males. Half lives of cocaine or BE were not statistically different between males and females (Mann-Whitney *U*-test; $P = 0.93$ for cocaine, $P = 0.99$ for BE). Half lives of cocaine and BE were strongly correlated (Spearman rank rho = 0.73; $P < 0.001$). Consequently, cocaine and BE were detectable in hair of former drug users after several months of abstinence. The calculated half-life of over 1 month for cocaine suggests that, assuming first order elimination, approximately 3-4 months have to pass for hair testing to become negative in the segment proximal to the scalp. This finding should be included when interpreting compliance with abstinence of former drug users, and indicates that caution should be exerted when evaluating potential breaches of abstinence

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J Anal Toxicol 2009 **33** (6) 343
Considerations on the utility of hair analysis for cocaine (Letter)
Hair analysis for drugs of abuse has been practiced in the field of forensic toxicology is long established. The FBI Laboratory has analyzed hair samples for cocaine and metabolites for over 2 decades. Most requests for analysis have involved criminal informants or subjects in public corruption cases when other, more traditional, toxicological specimens cannot be obtained. However, recent research findings that indicate that cocaine may be absorbed into hair *via* external contamination to a higher extent than had previously been believed. Therefore, the FBI Laboratory has suspended cocaine analyses in hair for most cases. In a report to the National Institute of Justice earlier in 2009, Ropero-Miller and Stout described several protocols to determine if hair collected from chronic users of cocaine could be distinguished from hair that was externally contaminated with cocaine hydrochloride. Their results suggest that cocaine may be incorporated into human hair *via* exterior contamination in concentrations that correspond to the amounts found in the hair of cocaine users

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Forensic Sci Int 2009 **190** (1-3) 16
Distribution of methamphetamine and amphetamine in drug abusers' head hair
The concentrations of methamphetamine (MA) and amphetamine (AP) in 2070 hair samples from MA abusers were statistically evaluated. Concentrations of MA and AP in hair were classified arbitrarily into three groups representing low, medium and high ranges and the metabolite-to-parent drug ratios of each group were investigated. The concentration ranges suggested here were also employed in the interpretation of five genuine cases. The low, medium and high ranges of MA were 0.5-4.2, 4.2-24.5 and 24.5-608.9 ng/mg and those of AP were 0.1-0.4, 0.4-1.7 and 1.7-41.4 ng/mg. The AP-to-MA ratios demonstrated large variation but a tendency to decrease as the MA ranges increased. This protocol produced very informative to presume the severity of MA abuse by an individuals and to provide law enforcement agencies with more understandable information. It might also assist a decision by the court regarding specific circumstances surrounding the drug-related crimes

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Forensic Sci Int 2009 **189** (1-3) 54

Detection of cocaine and its metabolites in urine using solid phase extraction-ion mobility spectrometry with alternating least squares

By employing solid phase extraction (SPE) coupled with ion mobility spectrometry (IMS) a reliable, alternative screening method for detection of cocaine and its metabolites, benzoylecgonine and cocaethylene in urine is described. Data analysis with alternating least squares (ALS) is employed to model IMS spectral datasets and separate the reactant ion peak from the product ion peaks. IMS has been employed as a screening device for drug and explosive detection for many years. When compared to similar methods, it has the advantages of atmospheric pressure operation, simple sample preparation, portability, fast analysis, and high sensitivity. When coupled with IMS, SPE decreases the detection limits of drug metabolites in urine while removing salts and other polar compounds that suppress ionization during the measurement. The IMS analysis time described is 20s which is much shorter than traditional chromatographic analysis. In addition, ALS further increases the sensitivity and selectivity of this method. The detection limits of benzoylecgonine and cocaethylene were 10 ng/ml and 4 ng/ml, respectively. Commercial adulteration of urine specimens did not affect the ability to detect cocaine metabolites after sampling the urine with SPE. This technique provides forensic chemists a viable approach for fast and simple drug screening

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Anal Chem 2009 **81** (16) 7081

Determination of cocaine in human plasma by selective solid-phase extraction using an aptamer-based sorbent

A highly selective solid-phase extraction (SPE) sorbent which exploits the properties of aptamers has been characterised. The selective extraction of cocaine from human plasma with an oligosorbent based on aptamers immobilized on a solid support was synthesized and tested. Anticocaine aptamers were immobilized to CNBr-activated Sepharose and an extraction technique was developed in pure media. Specific retention of cocaine on the oligosorbent was demonstrated and the capacity of the support was determined. The oligosorbent was evaluated with the selective extraction of cocaine from plasma at a concentration corresponding to the plasma concentration reached after an intake of a single dose of cocaine (0.4 mg/l). Extraction recovery around 90% was produced. Furthermore, interfering compounds that interfered with cocaine quantification when employing a standard SPE sorbent were not retained on the oligosorbent. This facilitated fast and reliable analyses of plasma samples with an estimated limit of detection of 0.1 µg/ml

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Anal Sci 2009 **25** (9) 1115

Determination of amphetamines in hair by GC/MS after small-volume liquid extraction and microwave derivatization

A simple and sensitive protocol for the determination of amphetamine drugs in hair is described. Results from the method employing small-volume extraction corresponds perfectly with those either from utilising the derivatization method or selected ion monitoring (SIM) detection. The technique was validated using four different amine drugs, including amphetamine, methamphetamine, methylenedioxy-amphetamine and methylenedioxy-methamphetamine. The detection limit for these drugs was approximately 50 +/- 7.5 pg/mg in hair and the intra-day and inter-day reproducibility were within 15% at most drug concentrations. Furthermore, the utility of the procedure in analyses of authentic hair samples taken from amphetamine abusers was examined. It demonstrated that the procedure is sufficient for the analysis of a trace amounts of amphetamines in human hair

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Rapid Commun Mass Spectrom 2009 **23** (23) 3772

Investigation of the identification point system adaptation in cocaine, benzoylecgonine and ecgonine methyl ester using a single quadrupole mass spectrometer

At present, no official criteria exist for drug identification using single quadrupole mass spectrometers although the European Union (EU) criteria for compound identification have been adopted. These criteria are evaluated with respect to the confirmation of cocaine and its metabolites by single quadrupole liquid chromatography/mass spectrometry (LC/MS) and problems are highlighted. Spiked samples, proficiency testing samples, certified reference materials and samples from real cases that had screened positive for cocaine derivatives by immunoassay were subjected to confirmation by LC/MS using single ion monitoring with in-source fragmentation. The EU criteria for compound identification were applied for the confirmation of cocaine, benzoylecgonine and ecgonine methyl ester. The use of the identification point (IP) system in spiked, proficiency testing samples and certified reference materials provided acceptable results in all cases while in some cases real positive samples did not provide acceptable results. Failure to meet the EU criteria was attributed to low fragmentation at the lower concentrations and the ion suppression effect while both factors affected compliance with the IP system. The identification of cocaine and its metabolites was considerably improved by using a combination of ammonium formate and formic acid as the LC mobile phase. It appears that poor in-source fragmentation in single quadrupole LC/MS and ion suppression may constitute a problem with drug identification when implementing the IP system in real samples, resulting in false negative results. Further investigation is needed for the use of such IP systems to be suitable for use in LC/MS methods

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Forensic Sci Int 2009 **189** (1-3) e41

Hair testing is superior to urine to disclose cocaine consumption in driver's licence regranting

Subjects with a history of cocaine abuse are required to undergo laboratory testing to verify both current and past abstinence from the drug before regranting of their driver's licence. However, identification of cocaine use employing only urinalysis could miss some cases because of the short elimination half-life of the drug. In addition, many abusers know how to time their cocaine consumption in such a way that they can foil the urinalysis and so have a series of negative urine tests. The use of hair testing has been emplyed to disclose sporadic cocaine consumption in seven subjects attending the Local Medical Commission to reobtain driver licence where there was consistant negative urinalysis. Even where there was one or two weekly negative urine screens over several months, all the subjects tested positive using hair testing for cocaine and benzoylecgonine, above the internationally recommended limit of quantification. These were 0.5 ng/mg and 0.05 ng/mg for cocaine and benzoylecgonine, respectively (concentration range for cocaine: 0.51-2.23 ng/mg hair; concentration range for benzoylecgonine: 0.08-1.70 ng/mg hair). The results vindicate the use of hair testing for cocaine in drug addicts and occasional abusers who apply for the regranting of driver licences in order to minimize social risk behaviour

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Forensic Sci Int 2009 **190** (1-3) 1

Mass and NMR spectroscopic characterization of 3,4-methylenedioxypyrovalerone: A designer drug with -pyrrolidinophenone structure

A drug variant of pyrovalerone MDPV was first seized in Germany in the year 2007 is the designer drug 3,4-methylenedioxypyrovalerone (MDPV). An investigation has been made of it using nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy. Structure analysis of the aliphatic part of MDPV was obtained by product ion spectroscopy of the immonium ion with *m/z* 126 produced following electron ionization, and by 1D ^{1}H and ^{13}C NMR spectroscopy. Additional two-dimensional NMR spectroscopy was utilised to confirm the structure of the alkyl side chain, and to deduce the methylenedioxy position in the aromatic ring

10 Hallucinogens

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Clin Toxicol 2009 **47** (6) 566

Bioanalytical and clinical evaluation of 103 suspected cases of intoxications with psychoactive plant materials

A bioanalytical and clinical study of intoxication cases suspected to be linked to abuse of plant-derived psychoactive substances was undertaken. Urinalysis was conducted on samples collected at emergency wards in Sweden from patients who either admitted or were suspected of ingestion of psychoactive plant materials. Bioanalysis employed liquid chromatography-tandem mass spectrometry for 10 plant-derived substances (atropine, dimethyltryptamine, ephedrine, harmaline, harmine, ibogaine, lysergic acid amide, psilocin, scopolamine, and yohimbine) and gas chromatography-mass spectrometry for asarone. Routine testing for illicit drugs was also performed. During a 4-year period, 103 urine samples collected from mainly young people (age range 13-52 years, median 19) were analysed. Of 53 cases where ingestion of any of the 11 plant-derived substances examined in this study was admitted or suspected, 41 (77%) were confirmed bioanalytically. Nine of the 11 substances under investigation

were detected, the exceptions being ibogaine and yohimbine. Psilocin, derived from the consumption of hallucinogenic mushrooms, was the most frequent substance and accounted for 54% of the cases. The most frquent means of drug acquisition (56%) was *via* purchase over the Internet. Patients using psychoactive plant materials were predominantly young and usually used the Internet for drug acquisition. Therefore, access to bioanalytical methods for detection of plant-derived psychoactive substances is important when providing a clinical toxicology service

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J Sep Sci 2009 **32** (19) 3301

Selective sample pretreatment by molecularly imprinted polymer for the determination of LSD in biological fluids

For the first time, a molecularly imprinted polymer (MIP) was synthesized by a noncovalent imprinting approach for the selective extraction of an illicit drug, LSD, from hair and urine samples. For the synthesis of MIP, an analog of LSD, was taken as a dummy template, methacrylic acid as a functional monomer, and ACN as a porogen solvent. The MIP was used for offline extraction before HPLC-MS analysis. By studying the interactions taking place between the LSD and the MIP, a selective procedure was established in organic media and applied to hair samples. By this way, 0.1 ng/mg of LSD was successfully detected in hair with 82% of extraction recovery. A low retention was also obtained on the control polymer (only 9%). This procedure was then modified to obtain a selective extraction in aqueous media for the determination of LSD in urine samples. The comparison with a conventional C18 clearly demonstrated the selectivity brought by the MIP to the determination of LSD in urine. LSD was easily detected in urine at only 0.5 ng/ml with 83% of extraction recovery on the MIP and 11% on the NIP. An LOQ of 0.2 pg/ml was estimated in urine samples.

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Forensic Sci Int 2009 **189** (1-3) 60

Manufacturing by-products from, and stereochemical outcomes of the biotransformation of benzaldehyde used in the synthesis of methamphetamine

Pseudoephedrine extracted from over the counter cold and flu medications has been the predominant source for the clandestine synthesis of methamphetamine in Australia. However, pseudoephedrine from these sources has become much more difficult to obtain following the restrictions which have recently been introduced on the sale of these products. Consequently, clandestine chemists have resorted to other sources of the required chemical precursors. Recently, a drug raid (Adelaide, January 2008) resulted in the seizure of a reaction mixture that indicated a unusual approach. This was the product of the fermentation of glucose by yeast in the presence of benzaldehyde to give 1-hydroxy-1-phenylpropanone, also known as l-phenylacetylcarbinol (l-PAC), a known precursor to ephedrine and pseudoephedrine and hence methamphetamine. An investigation was conducted into this process with the aim of determining the characteristic reaction by-products associated with methamphetamine produced in this manner. Also determined was the stereochemical selectivity of the fermentation reaction and the stereochemistry of the subsequent reaction products, ephedrine and pseudoephedrine, and the final methamphetamine

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J Forensic Sci 2009 **54** (6) 1485

Morbidity involving the hallucinogenic designer amines MDA and 2C-I

A case is presented of a 39-year-old woman who suffered severe debilitation because of a hemorrhagic stroke in the context of substance abuse. The patient presented to the emergency room with rapidly diminishing mental status, hypertension, and vasoconstriction; her friends provided a history of ingestion of cocaine, 3,4-methylenedioxymethamphetamine (MDMA), and 2C-I, a novel designer amine. A multi-targeted LC-MS/MS method for sympathomimetic amines and related drugs in urine detected and quantified 2C-I and MDA, while ruling out MDMA. The cause of the stroke was determined to be an underlying cerebrovascular abnormality termed moyamoya, secondary to substance abuse. In clinical laboratories, gas chromatography-mass spectrometry or liquid chromatography-tandem mass spectrometry (LC-MS/MS) confirmation of a positive amphetamine immunoassay is usually directed only towards amphetamine, methamphetamine, MDMA and MDA. This report demonstrates the utility of testing for a wider menu of compounds using LC-MS/MS in order to better characterize the prevalence and toxicities of novel amines such as 2C-I.

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Analyst 2009 **134** (8) 1565

Development of a piezoelectric sensor for the detection of methamphetamine

A synthetic receptor for methamphetamine has been employed in the development of a piezoelectric sensor by utilising a computationally designed molecularly imprinted polymer (MIP). Immobilisation of the MIP onto the gold sensor surface was investigated with several different protocols. The detection limit of the MIP sensor for methamphetamine was as low as 1 µg/ml. The effect of the addition of poly(vinyl acetate) (PVA) on the pre-polymerisation mixtures, which increases the porosity of the polymer layer, was also studied using atomic force microscopy. PVA appeared to affect both the porosity and the binding kinetics of the polymers prepared in dimethylformamide (DMF). On the other hand, no apparent effect on porosity and binding kinetics was noted when polymers were prepared in diglyme. Furthermore, PVA did not appear to improve the amplitude of the sensor response. The sensor described in this work has excellent recognition ability in aqueous solutions. Consequently, it may be an useful starting point for the development of a commercial device for fast, on-site or road-side testing of drugs of abuse in body fluids such as saliva

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J Anal Toxicol 2009 **33** (6) 332

Concentration-time profiles of -hydroxybutyrate in blood after recreational doses are best described by zero-order rather than first-order kinetics

Gamma-hydroxybutyrate (GHB) is a recreational drug with a short plasma elimination half-life ($t_{1/2}$) dtermined at about 30-50 min. This represents a terminal half-life. Consequently, it might not necessarily apply where large doses (abuse) are consumed. Clinical studies with sodium oxybate (sodium salt of GHB) indicate that zero-order rather than first-order kinetics are more satifactory to describe post-peak concentration-time (C-T) profiles. The case of a 23-year-old male discovered unconscious by the police and with a blood sample containing 100 mg/l GHB and 0.14 g% ethanol. After regaining consciousness, the man admitted consuming alcohol about 6 h previously. He explained the presence of GHB by claiming that his drink must have been spiked. However, the police wanted to know how much GHB had been administered to account for the man's clinical condition. By employing a back-calculation for 6 h and assuming a GHB half-life of 40 min suggests a very high concentration in blood of approximately 900 mg/l, which would probably have proven fatal. However, back-calculating using zero-order kinetics and a proposed elimination rate of 18 mg/l per hour suggets a GHB concentration of 208 mg/l which is far more realistic. Consequently, toxicologists should not arbitrarily apply the principles of first-order kinetics after abuse doses of drugs when zero-order or saturation kinetics (Michaelis-Menten) are more appropriate

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Addiction 2009 **104** (12) 2041

Do 9-tetrahydrocannabinol concentrations indicate recent use in chronic cannabis users?

The aim was to quantify blood Δ^9-tetrahydrocannabinol (THC) concentrations in chronic cannabis users over 7 days of continuous monitored abstinence. Twenty-five frequent, long-term cannabis users resided on a secure clinical research unit at the US National Institute on Drug Abuse under continuous medical surveillance to prevent cannabis self-administration. Whole blood cannabinoid concentrations were determined by two-dimensional gas chromatography-mass spectrometry. Nine chronic users (36%) had no measurable THC during 7 days of cannabis abstinence; 16 had at least one positive THC > or = 0.25 ng/ml, but not necessarily on the first day. On day 7, 6 full days after entering the unit, six participants still displayed detectable THC concentrations [mean +/- standard deviation (SD), 0.3 +/- 0.7 ng/ml] and all 25 had measurable carboxy-metabolite (6.2 +/- 8.8 ng/ml). The highest observed THC concentrations on admission (day 1) and day 7 were 7.0 and 3.0 ng/ml, respectively. Interestingly, five participants, all female, had THC-positive whole blood specimens over all 7 days. Body mass index did not correlate with time until the last THC-positive specimen (n = 16; r = -0.2; P = 0.445). Substantial whole blood THC concentrations persist multiple days after drug discontinuation in heavy chronic cannabis users. It is currently unknown whether neurocognitive impairment occurs with low blood THC concentrations, and whether return to normal performance, as documented previously following extended cannabis abstinence, is accompanied by the removal of residual THC in brain. These findings also may impact on the implementation of *per se* limits in driving under the influence of drugs legislation.

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Yakugaku Zasshi 2009 **129** (8) 975

Chemical and DNA analyses for the products of psychoactive plant, *Voacanga africana* (Japanese, English Abstract)

Voacanga africana (Apocynaceae) is a small tropical African tree. The root bark and seeds of this tree contain a number of alkaloids. These include ibogaine (a hallucinogenic/aphrodisiac compound in bark), tabersonine (a major constituteent of seeds) and other *Voacanga* alkaloids, traditionally used in Africa for religious purposes. Products containing this plant (root bark and seeds) have been available on the drug market recently with the expectation of hallucinogenic/aphrodisiac effects. Quantitative analyses of these alkaloids in products and botanical specimens have not been described. A simultaneous analytical method has been developed employing LC/MS for the *Voacanga* alkaloids including ibogaine and tabersonine in commercial products of *V. africana*. Furthermore, the botanical origins of these products were investigated by DNA analyses. Following LC/MS analyses, the products were classified into two chemical types; an ibogaine-type and a tabersonine-type. Samples of the ibogaine-type contain ibogaine (0.05-0.6%) and other *Voacanga* alkaloids; voacamine, voacamidine and voacangine, whereas those of the tabersonine-type mainly contain tabersonine (0.6-1.6%). Chloroplast DNA sequence analyses of the *trn*L-F region indicated that most of the products were produced from *V. africana* or closely related plants. Four genotypes were identified based on nucleotide sequence of the *trn*L-F IGS region. The described procedures for chemical and DNA analyses should facilitate investigations of the trends in the distribution of the products of *V. africana*

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J Anal Toxicol 2009 **33** (7) 366

Methylenedioxymethamphetamine-related deaths in Taiwan: 2001-2008

One of most popular drugs on the "club" scene in Taiwan is methylenedioxymethamphetamine (MDMA). Toxicological data produced from the 59 fatalities tested positive for MDMA during the period of January 2001 to December 2008 were studied. Ketamine was identified in 28 of these cases, indicating the popularity of this drug in Taiwan. The annual number of deaths in each of the 8 years in this period was 4, 7, 9, 14, 8, 9, 2, and 6, respectively. Of these 59 deaths, 39 (66.1%) were men, and the mean, median, and range of ages were 24.6, 23, and 14-46, respectively. Pathological examination indicated that fatalities resulted from acute intoxication (40) and mechanical injury (19, including 3 by hanging and 2 by drowning). Manners of death were adjudged as accidental (44) homicidal (6) suicidal (7) and undetermined (2). Postmortem whole blood was analyzed by gas chromatography-mass spectrometry with a limit of quantitation at 0.05 µg/ml for both MDMA and MDA. Where MDMA acute intoxication was ruled as the cause of death, the mean, median, and range of MDMA concentrations were 4.75, 2.60, and 0.12-40.41 µg/ml. MDA was discovered in 30 of these 40 cases with the following mean, median, and range data: 0.19, 0.13, and 0.05-1.81 µg/ml. Corresponding data for MDMA and MDA in the remaining 19 MDMA-related deaths were significantly lower: 1.25, 0.97, 0.08-3.05 and 0.11, 0.09, 0.06-0.24 µg/ml, respectively

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J Forensic Sci 2009 **54** (5) 1176

Combined dextromethorphan and chlorpheniramine intoxication in impaired drivers

Dextromethorphan is a nonprescription antitussive which has been gaining in popularity as an abused drug, because of the hallucinogenic, dissociative, and intoxicating effects it produces at high doses. This report describes a series of eight drivers arrested for driving under the influence of the combined effects of dextromethorphan and chlorpheniramine, and a further four drivers under the influence of dextromethorphan alone. In the combined dextromethorphan/chlorpheniramine cases, blood dextromethorphan concentrations ranged from 150 to 1220 ng/ml (n = 8; mean 676 ng/ml, median 670 ng/ml), and chlorpheniramine concentrations ranged from 70 to 270 ng/ml (n = 8; mean 200 ng/ml, median 180 ng/ml). The four cases without chlorpheniramine present had blood dextromethorphan concentrations between 190 and 1000 ng/ml (mean 570 ng/ml, median 545 ng/ml). Some drivers had therapeutic concentrations of other drugs present. Drivers generally displayed symptoms of central nervous system (CNS) depressant intoxication, and there was gross evidence of impairment in their driving, including weaving, leaving the lane of travel, failing to obey traffic signals, and involvement in collisions. Drug recognition expert opinions confirmed that the subjects were under the influence of a drug in the CNS-depressant category

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Rapid Commun Mass Spectrom 2009 **23** (22) 3543

Rapid drug detection in oral samples by porous silicon assisted laser desorption/ionization mass spectrometry

The demand for analysis of oral fluid for illicit drugs has arisen with the increased adoption of roadside testing, particularly in countries where changes in legislation allow random roadside testing of drivers for the presence of a palette of illicit drugs such as methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA) and Δ^9-tetrahydrocannabinol (THC). Oral samples are currently tested for such drugs at the roadside using an immunoassay-based commercial test kit. Positive roadside tests are sent for confirmatory laboratory analysis, traditionally by means of gas chromatography/mass spectrometry (GC/MS). We present here an alternative rapid analysis technique, porous silicon assisted laser desorption/ionization time-of-flight mass spectrometry (pSi LDI-MS), for the high-throughput analysis of oral fluids. This technique alleviates the need for sample derivatization, requires only sub-microliter sample volumes and allows fast analysis (of the order of seconds). In this study, the application of the technique is demonstrated with real samples from actual roadside testing. The analysis of oral samples resulted in detection of MA and MDMA with no extraction and analysis of THC after ethyl acetate extraction. We propose that, subject to miniaturization of a suitable mass spectrometer, this technique is well suited to underpin the deployment of oral fluid testing in the clinic, workplace and on the roadside

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J Forensic Sci 2009 **54** (6) 1341

Illegal route estimation of the seized illicit drug, methamphetamine, by the comparison of striation marks on plastic packaging films

In Japan, the most common illicit drug is methamphetamine. It is possible to trace the origin of this drug by analyzing its organic and inorganic impurities and/or byproducts using several methods, such as GC, GC/MS, and inductively coupled plasma-mass spectrometry (ICP-MS). As reported here, one other method includes comparison of the striation lines of polymer sheet layers from packaging using a polarized light method. Other alternative methods include analyzing the heat sealer pattern, layer thickness surface characteristics, and/or components of polymer sheet layers using infrared spectroscopy. Several of these alternative methods were used to analyze the origins of 29 packages confiscated from three regions over a 1000 km distance in Japan. Results indicated that packages seized from different regions had some polymer sheet layers which contained striation lines and heat sealer patterns that were similar.

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Addiction 2009 **104** (12) 2057

Content of Ecstasy in the Netherlands: 1993-2008

The present paper outlines the results of analyses carried out on the content of tablets sold as Ecstasy, collected in the Netherlands by the Drugs Information Monitoring System (DIMS) from January 1993 to December 2008. During a period of 16 years, the DIMS analysed the content of 33 006 tablets sold as Ecstasy that were handed in by numerous individual (potential) substance users. The DIMS results were compared with the results from various seized tablets to determine whether the DIMS is a monitor of the Ecstasy consumer market. The DIMS system appears to be a market monitor that gives an accurate reflection of what is actually available on the hidden Dutch Ecstasy market. During 16 years of monitoring, the purity [tablets containing only 3,4-methylenedioxymethamphetamine (MDMA)] was lowest around 1997. During this time-period many tablets contained other substances in addition to or instead of MDMA [e.g. 3,4-methylene-dioxyamphetamine (MDA), 3,4-methylene-dioxyethylamphetamine (MDEA) and *N*-methyl-a-(1,3-benzodixol-5-yl)-2-butamine (MBDB), amphetamine and caffeine]. From 1998 to 2008, the number of high-dose tablets (> or = 106 mg MDMA per tablet) gradually increased. The same holds true for the proportion of tablets that contained only MDMA, reaching the highest levels in 2000 and 2004. After 2004, the purity of Ecstasy tablets decreased again, caused mainly by a growing proportion of tablets containing *meta*-chlorophenylpiperazine (*m*CPP). The DIMS results provide valuable qualitative information on the content of Ecstasy tablets in the Netherlands, and its changes throughout the years. Moreover, the results were used for national and international risk assessments and important warning and prevention activities.

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J Forensic Sci 2009 **54** (6) 1261

The stable isotope ratios of marajuana. II. Strontium isotopes relate to geographic origin

Effectively addressing marijuana trade is aided by understanding marijuana

geographic sources. We analyzed the $^{87}Sr/^{86}Sr$ of marijuana samples grown in 79 counties across the United States to determine if a primary geologic signal is retained in marijuana, which could therefore be useful for geographic sourcing. The marijuana results were compared with modeled bedrock $^{87}Sr/^{86}Sr$ values based on ^{87}Rb decay rates and a generalized geologic map of the U.S.A. A significant correlation was observed between marijuana $^{87}Sr/^{86}Sr$ and modeled bedrock $^{87}Sr/^{86}Sr$. Although values clustered near the 1:1 relationship, there was a predominance of positive anomalies, perhaps attributable to carbonate bedrock. A small number of negative anomalies were also observed, which were generally associated with granitic bedrocks. These results suggest that strontium isotopes in marijuana record the geographic origins of marijuana, and that refinement of the base strontium map (or strontium isoscape) and improved understanding of other strontium sources would be productive.

11 Narcotics

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J Forensic Sci 2009 **54** (5) 1181
Morphine concentrations in stomach contents of intravenous opioid overdose deaths
Death caused by heroin overdose is almost always the result of intravenous injection of the drug in Australia. We briefly describe a case where a heroin overdose was initially thought to be the result of oral ingestion of the drug, primarily as a result of higher concentrations of morphine in stomach contents than in blood. During the subsequent criminal trial and investigation, however, the issue of the entero-hepatic circulation of morphine was raised as a possible reason for the presence of morphine in the stomach contents. In this study, we report on the distribution of opioids in blood, stomach contents, urine, liver, and bile in 29 deaths caused by intravenous heroin overdose. The mean total and free blood morphine concentrations were 0.60 and 0.32 mg/l, respectively, and the mean stomach contents total morphine concentration was 1.16 mg/kg. All cases had detectable morphine in the stomach contents, and 24 of 29 cases (83%) had higher concentrations of total morphine in stomach contents than in blood. The mean total morphine concentration in bile was *c*. 100 times that in blood, and the liver total morphine concentration averaged twice that of blood levels. We conclude that the entero-hepatic circulation of morphine and subsequent reflux of duodenal contents back into the stomach can result in the deposition of morphine in gastric contents. Consequently, the relative levels of opioids in blood and stomach contents cannot be used to determine the site of administration of the drug

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J Anal Toxicol 2009 **33** (6) 310
Validation of an enzyme-linked immunosorbent assay screening method and a liquid chromatography-tandem mass spectrometry confirmation method for the identification and quantification of ketamine and norketamine in urine samples from Malaysia
An ELISA and a liquid chromatography-tandem mass spectrometry (LC-MS-MS) confirmation method were developed and validated for the identification and quantitation of ketamine and its major metabolite norketamine in urine samples. The Neogen ketamine microplate ELISA kit has been validated to incorporate an assessment of the dose-response curve, intra- and interday precision, limit of detection (LOD), and cross-reactivity. It has been optimized in respect of sample and enzyme conjugate volumes and the sample preincubation time before addition of the enzyme conjugate. The sensitivity and specificity were derived from a comparison with the results from the validated LC-MS-MS confirmation method. An LC-MS-MS procedure was developed and validated in respect of LOD, lower limit of quantitation (LLOQ), linearity, recovery, intra- and interday precision, and matrix effects. The dose-response curve of the ELISA was a typical S-shaped binding curve, with a linear portion of the graph observed between 25 and 500 ng/ml for ketamine. The cross-reactivity of 200 ng/ml norketamine to ketamine was 2.1%, and no cross-reactivity was detected with 13 common drugs tested at 10,000 ng/ml. The ELISA LOD was calculated to be 5 ng/ml. Both intra- (n = 10) and interday (n = 50) precisions were below 5.0% at 25 ng/ml. The LOD for ketamine and norketamine was calculated statistically to be 0.6 ng/ml. The LLOQ values were also calculated statistically and were 1.9 ng/ml and 2.1 ng/ml for ketamine and norketamine, respectively. The test linearity was 0-1200 ng/ml with correlation coefficient (r^2) > 0.99 for both analytes. Recoveries at 50, 500, and 1000 ng/ml range from 97.9% to 113.3%. Intra- (n = 5) and interday (n = 25) precisions between extracts for ketamine and norketamine were excellent (< 10%). Matrix effects analysis demonstrated a mean ion suppression of 5.7% for ketamine and an average ion enhancement of 13.0% for norketamine for urine samples collected from six individuals. A comparison of ELISA and LC-MS-MS results established a sensitivity, specificity, and efficiency of 100%. The results suggest that a cutoff value of 25 ng/ml ketamine in the ELISA screen is particularly applicable and reliable for urinalysis in a forensic toxicology setting. In addition, both ketamine and norketamine were detected in all 34 urine samples collected from individuals socializing in pubs by the Royal Malaysian Police. Ketamine concentrations detected by LC-MS-MS ranged from 22 to 31,670 ng/ml, and norketamine concentrations ranged from 25 to 10,990 ng/ml. The concentrations of ketamine and norketamine detected in the specimens are suggestive of ketamine abuse

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J Anal Toxicol 2009 **33** (7) 345
Determination of heroin and its main metabolites in small sample volumes of whole blood and brain tissue by reversed-phase liquid chromatography-tandem mass spectrometry
The quantitative analysis of heroin and its major metabolites 6-acetylmorphine, morphine, morphine-3-glucuronide and morphine-6-glucuronide in blood and brain tissue has been achieved with high-performance liquid chromatography-tandem mass spectrometry (LC-MS-MS) employing 0.1ml samples. The protocol was validated by analysis of heroin and its metabolites in samples from heroin treated mice. Ice-cold acidic buffer containing sodium fluoride was immediately added to blood and brain homogenate samples. Sample preparation was accomplished by protein precipitation in an ice-bath using a mixture of ice-cold acetonitrile and methanol. The supernatant was evaporated to dryness, reconstituted with mobile phase, and injected into the chromatographic system. Separation was performed on a Xterra® C18 column with gradient elution. The MS analysis was achieved in positive ion mode and multiple reaction monitoring (MRM) was employed for drug quantification. The limits of quantification for the different opiates ranged from 0.0007 to 0.02 mg/l in blood and from 0.002 to 0.06 μg/g in brain tissue. Day-to-day relative standard deviation varied from 3.1 to 14.5%, and within-day variation ranged from 2.1 to 11.4%. Recoveries were between 80 and 111%. Heroin was found to be more stable in brain tissue than in blood

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Talanta 2009 **79** (3) 845
Simple and rapid voltammetric determination of morphine at electrochemically pretreated glassy carbon electrodes
Electrochemical pretreatment of a glassy carbon electrode (GCE) which was treated by anodic oxidation at 1.75 V, following potential cycling in the potential range from 0 V to 1.0 V *vs*. Ag/AgCl reference electrode was employed as the basis of a method for the simple and rapid detection of morphine. The sensitivity for morphine detection was greatly increased and the detection limit was 0.2 μM. The reproducibility of the voltammetric measurements was usually less than 3% RSD for six replicate measurements. Furthermore, this technique could readily differentiate morphine from codeine. The electrochemical detection of a urine sample spike with morphine was achieved with satisfactory results

12 Forensics

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Forensic Sci Int 2009 **189** (1-3) 82
Determination of pesticides in postmortem blood and bone marrow of pesticide treated rabbits
Forensic toxicological analyses have traditionally employed blood, body fluids, and certain organs in investigations of deaths due to intoxication. However, in some situations, such as in exhumation cases, putrefaction and contamination make proper sampling from tissues impossible. In such instances, bone marrow might be useful as an alternative specimen since it is a potential depot for drugs. In order to determine the diagnostic value of toxicological analysis of bone marrow in exhumation cases, analyses were made of pesticides in postmortem and putrefied bone marrow of treated rabbits. Out of thirteen rabbits, a 110 mg/kg dose of endosulfan was orally given to six through a gavage tool, and a 2500 mg/kg dose of diazinon was given to six using the same method. One rabbit was not treated with anything and served as a control sample. Venous blood, liver, lung, kidney, brain, and bone marrow samples were obtained either just after spontaneous death or cervical dislocation. Subsequently, the rabbits were buried in soil for 1 month then exhumed and putrefied viscera and

bone marrow sampled. Blood and tissue samples underwent solvent extraction and solid phase extraction, and then the samples were analyzed by GC-MS. Mean residue levels of diazinon in early postmortem samples were 85 mg/kg, 71 mg/kg, 23 mg/kg, 21 mg/kg, 19 mg/kg, and 0.4 mg/l in the liver, bone marrow, kidney, lung, brain, and blood, respectively. Mean residue levels of diazinon in the putrefied body were 3327 mg/kg in putrefied viscera and 1783 mg/kg in the bone marrow. Mean residue levels of endosulfan isomers and metabolites in early postmortem samples (blood, liver, lung, kidney, brain, and bone marrow) were 0.46 mg/kg (endosulfan sulfate), 0.32 mg/kg (α and β isomers of endosulfan), and 0.14 mg/kg (endosulfan ether) while the same levels were 0.26 mg/kg (endosulfan sulfate), 0.24 mg/kg (α and β isomers of endosulfan), and 0.1 mg/kg (endosulfan ether) in putrefied samples (putrefied bone marrow and putrefied viscera). Consequently, it is possible that cause of death may be determined as acute pesticide poisoning by toxicological analysis of samples from bone marrow and putrefied viscera in exhumation cases

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J Anal Toxicol 2009 **33** (7) 389

Lethal poisoning with ethiofencarb and ethanol

Due to their reversible acetylcholinesterase inhibition and relative inability to cross the blood-brain barrier, carbamate compounds including ethiofencarb are generally less toxic than organophosphorus insecticides. In general, ethiofencarb is regarded to be of low toxicity (LD_{50} > 200 mg/kg). On-the-other-hand, severe poisoning and death are not uncommon. Surprisingly, no analyses of ethiofencarb and its metabolites in human postmortem whole blood appear in the literature. A case report of fatal ethiofencarb intoxication is described where quantitative analysis of ethiofencarb and its metabolites in ante- and postmortem blood was accomplished. Furhtermore, postmortem urine was collected and analyzed. The case was that of a 56-year-old man who worked as a gardener and was found in poor condition seated in his car. He had been vomiting. He was admitted to the local hospital approximately 1 h later. On admission, he was conscious but unable to speak clearly. His condition deteriorated and he developed severe pulmonary edema. Resuscitation with atropine and adrenaline were attempted. However, he died approximately 3 h following admission. Analysis of postmortem peripheral blood indicated 0.12 g/100 ml ethanol, 26.4 mg/l ethiofencarb, 37.9 mg/l ethiofencarbsulfoxide, and 0.9 mg/l ethiofencarbsulfone. Ethanol (0.26 g/100 ml), ethiofencarb, ethiofencarbsulfoxide, and ethiofencarbsulfone were also identified in urine

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Biomed Chromatogr 2009 **23** (11) 1217

Ion chromatography tandem mass spectrometry for simultaneous confirmation and determination of indandione rodenticides in serum

This paper describes a simple method for the simultaneous determination and confirmation of the indandione rodenticides in serum. After samples were extracted with 10% (v/v) methanol in acetonitrile and cleaned by solid-phase extraction, chromatographic separation was performed on an IonPac AS11 analytical column (250 x 4.0 mm) using gradient KOH eluent with 10% (v/v) methanol as organic modifier. Confirmation was depended on the extensive fragmentation of the indandione molecule under MS/MS conditions which provides sufficient structural information. Quantification was performed by negative electrospray ionization in multiple reaction monitoring mode. All the method parameters were validated. It was confirmed that this method could be used in clinical diagnosis and forensic toxicology

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Anal Bioanal Chem 2009 **394** (8) 2151

Surface plasmon resonance immunosensor for cortisol and cortisone determination

Real-time detection of cortisol and cortisone levels in urine and saliva samples has been achieved with a surface-plasmon-resonance-based immunosensor. The technique developed here is simple, rapid, economic, sensitive, robust, and reproducible due to the special features of the polycarboxylate-hydrogel-based coatings employed for the antibody immobilization. The sensor surface demonstrates a high level of stability during repeated regeneration and affinity reaction cycles. The immunosensor shows high specificity for cortisol and cortisone. In addition, no significant interferences from other steroids with a similar chemical structure were noted. The suitability of the hydrogel coating for the elimination of nonspecific binding is also assessed. A good correlation is provided between the results obtained with the sensor and the reference liquid chromatography/tandem mass spectrometry method for the analysis of cortisol and cortisone in urine and saliva samples. Standard curves for the analysis of cortisol and cortisone in saliva and urine are characterized by a detection limit less than 10 μg/l which is sufficiently sensitive for both clinical and forensic use

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Forensic Sci Int 2009 **190** (1-3) 9

Quantitative structure-activity relationship (QSAR) methodology in forensic toxicology: Modeling postmortem redistribution of structurally diverse drugs using multivariate statistics

Postmortem redistribution (PMR) is a multifaceted process. It may produce inaccuracies for drug concentrations reported by forensic toxicologists. Quantitative structure-activity relationship (QSAR) has been investigated as an effective tool to determine the ability of drugs to redistribute across tissue barriers during postmortem period on the basis of their molecular, physicochemical and structural properties. In this respect, multivariate data analysis (MVDA) was applied to a set of 77 structurally diverse drugs. PMR data expressed by the central:peripheral concentration ratio (C:P ratio) was obtained from the literature. An adequate and robust QSAR model (r^2 = 0.65, Q^2 = 0.56, RMSEE = 0.34) was determined for 59 (77%) out of 77 drugs. Whereas the derived QSAR protocol demonstrated limited applicability, it produced an informative illustration of the contributing molecular, physicochemical and structural properties in PMR process. Drugs with strong basic properties and enhanced molecular size, flexibility, lipophilicity and number of halogens facilitated increased PMR. Due to the myriad processes involved in the PMR process, additional QSAR research needs to focus on structurally related drugs to produce more specific models. These could serve as alternative tools to evaluate PMR for different chemical classes

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J Chromatogr B 2009 **877** (25) 2652

Rapid and quantitative screening method for 43 benzodiazepines and their metabolites, zolpidem and zopiclone in human plasma by liquid chromatography/mass spectrometry with a small particle column

Benzodiazepines and their pharmacologically related drugs, zolpidem and zopiclone are routinely prescribed in clinical practice. However, they are also abused in cases of crime, suicide and drug-facilitated sexual assault. A rapid and quantitative screening method has been developed for 43 benzodiazepines, their metabolites, zolpidem and zopiclone in human plasma by liquid chromatography/mass spectrometry with a small particle column. All drugs were proficiently separated within 12 min by employing combined scan and selected ion recording (SIR) mode. The calibration curves of most drugs were linear in the concentration range 0.5-250 ng/ml with correlation coefficients exceeding 0.99. Within-day precisions (RSD, %) of this method were 1.8-15.6% (10 ng/ml) and 0.6-10.1% (100 ng/ml) and between-day precisions (RSD, %) were 1.6-16.9% (10 ng/ml) and 0.6-16.7% (100 ng/ml). The average recoveries were 70.1% (10 ng/ml) and 87.1% (100 ng/ml). The limit of detection ranged from 0.2 to 8.0 ng/ml in 37 drugs and was below 0.2 ng/ml in 6 drugs. The developed procedure is sensitive and rapid. It should facilitate forensic and clinical toxicological analyses

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J Anal Toxicol 2009 **33** (6) 294

Simultaneous measurement of indandione-type rodenticides in human serum by liquid chromatography-electrospray ionization-tandem mass spectrometry

Indandione-type rodenticides may be accidentally ingested. Therefore, adequate assays are important for diagnosis and treatment. However, current assays do not possess effective capacity for the simultaneous analysis of several indandiones and particularly the isomers. The aim of this study was to develop a novel and selective procedure for the simultaneous determination of indandione-type rodenticides (diphacinone, chlorophacinone, valone, and pindone) in human serum by liquid chromatography-electrospray ionization-tandem mass spectrometry. Following addition of an internal standard, the sample was extracted with 10% methanol in acetonitrile and cleaned by solid-phase extraction (SPE). The analytes were separated on a C_{18} rapid column and infused into an ion trap mass spectrometer in the negative electrospray ionization mode. The multiple-reaction monitoring ion pairs were *m/z* 339 → 167, *m/z* 373 → 201, *m/z* 229 → 145, *m/z* 229 → 172, and *m/z* 307 → 161 for diphacinone, chlorophacinone, valone, pindone, and IS, respectively. Recoveries were between 81.5 and 94.6%, and the limits of quantification were 0.2 to 0.5 ng/ml. Intra- and interday RSDs were less than 7.9 and

11.5%, respectively. The assay was linear in the range of 0.5-100.0 ng/ml with coefficients of determination (r^2 > 0.99) for all rodenticides. The described procedure facilitates the unambiguous qualification and quantification of indandiones in both clinical and forensic specimens

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J Anal Toxicol 2009 **33** (7) 351

Cocaine and opiates use in pregnancy: Detection of drugs in neonatal meconium and urine

A case of a newborn with symptoms of hyperexcitability was investigated. Enzyme multiplied immunoassay attested to the use of drugs during pregnancy by the mother. Samples of the newborn's urine and meconium were analysed to follow the evolution in the distribution of cocaine and opiates during the days following birth. Urinalysis screening was achieved using an immunoassay technique and the confirmwith gas chromatography-mass spectrometry (GC-MS). A GC-MS technique for simultaneous determination of cocaine, benzoylecgonine, codeine, morphine, and 6-acetylmorphine in meconium is described. GC-MS results from urine and meconium indicated consumption of cocaine and codeine during pregnancy. In addition, the levels of drugs declined gradually and were completely eliminated by the third day

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J Anal Toxicol 2009 **33** (6) 336

Toxicological findings in two planned complex suicide cases: Ingestion of petroleum distillates and subsequent hanging

Two fatal cases of planned complex suicide by two male individuals, 86 and 51 years old, involving ingestion of petroleum distillates and hanging is described. During autopsy of both cases, the intense odor of petroleum distillates alerted authorities to the possibility of ingestion. Toxicological screening and quantitation of these compounds was initially performed by gas chromatography with flame-ionization detection and confirmation was determined by employing gas chromatography-mass spectrometry in total ion chromatogram mode after liquid-liquid extraction of biological samples using a previously published analytical procedure. Case 1: In addition to therapeutic concentrations of citalopram, diesel fuel No. 2 concentrations were < 5 mg/l heart blood and 18,160 mg/l gastric content (total amount 6356 mg). Case 2: Therapeutic concentrations of nordiazepam, oxcarbazepine, ibuprofen, and metamizol were found in blood and xylene (mixture of isomers) concentrations were 0.3 mg/l in heart blood and 0.1 mg/l in gastric content (total amount 0.006 mg); ethanol (1.12 g/l). The medical examiners in both instances reported the cause of death as hanging. In addition, examination of the scenes and the anatomopathological and toxicological data, the manners of death were determined to be planned complex suicide. Toxicologists should test for petroleum distillates when there is a suspicion of ingestion of these products due to the odor observed at the scene of death and/or during autopsy. These toxicological investigations could assist in determine the manner of death and the medicolegal interpretation

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Electroanalysis 2009 **21** (19) 2165

The electrochemical behavior of nitrazepam at a screen-printed carbon electrode and its determination in beverages by adsorptive stripping voltammetry

The cyclic voltammetric behavior of nitrazepam was investigated at screen-printed carbon electrodes over the range -1.5 V to +1.5 V. Two reduction peaks were observable on the negative scan, at -0.7 V, and -1.2 V using pH 6 buffer. On the return scan a single oxidation peak was obtained at -0.05 V. For quantitative analysis of beverages, we developed an anodic adsorptive stripping voltammetric method which required only dilution with buffer. The identification of nitrazepam and flunitrazepam could be achieved using cyclic voltammetry

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J Chromatogr B 2009 **877** (25) 2624

Electrospray ionization tandem mass spectrometric determination of monomethylarsonic acid and dimethylarsinic acid after adduct formation with citric acid

Monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) are metabolites of inorganic arsenic species which are subsequently excreted into urine. The simultaneous determination of MMA^V and DMA^V has been achieved by a simple, rapid and sensitive method employing electrospray ionization tandem mass spectrometry (ESI-MS-MS). MMA^V and DMA^V in a sample were allowed to react with citric acid (CiA) and adduct compounds extracted with isoamyl alcohol (IAA). An aliquot (1 µl) of the IAA layer was injected directly into the ESI-MS-MS instrument and was detected within 1 min. Quantification was accomplished with selected reaction monitoring for MMA^V and DMA^V as follows: $[MMAH + 2CiA - {}^3H_2O]^+ \rightarrow [MMAH + CiA - {}^2H_2O]^+$ $[DMAH + CiA + MeOH - {}^2H_2O]^+ \rightarrow [DMAH + MeOH - H_2O]^+$ where MMAH and DMAH denote the protonated forms of MMA^V and DMA^V, and MeOH denotes methanol (carrier liquid in ESI-MS-MS). The procedure was verified for the analysis of urine samples. The limit of detection of As was 0.3 µg/l for MMA^V and 0.6 µg/l for DMA^V using 10 µl of sample solution. Results were obtained in <10 min with a linear calibration range of 3-100 µg/l. Inorganic arsenic compounds (and other organic arsenic compounds) present in urine did not interfere with the detection of MMA^V and DMA^V. Concentrations of MMA^V and DMA^V in the reference urine SRM 2670a were evaluated after partial purification, and those in urine of a patient treated with As_2O_3 were measured following dilution

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Anal Bioanal Chem 2009 **394** (8) 2039

Detection of illicit substances in fingerprints by infrared spectral imaging

FTIR and Raman spectroscopy may be employed to simultaneously image a latent fingerprint and detect exogenous substances deposited within it. Such substances might include drugs of abuse or traces of explosives or gunshot residue. Spectral searching algorithms were investigated for their efficacy in finding targeted substances deposited within fingerprints. "Reverse" library searching, where a large number of possibly poor-quality spectra from a spectral image are compared with a small number of high-quality reference spectra, provides issues for common search algorithms as they are routinely implemented. From a range of algorithms including conventional Euclidean distance searching, the spectral angle mapper (SAM) and correlation algorithms provided the best results when used with second-derivative image and reference spectra. All procedures employed resulted in poorer performances with first derivative and undifferentiated spectra. In a search against a caffeine reference, the SAM and correlation methods were capable of correctly ranking a set of 40 confirmed but poor-quality caffeine spectra at the top of a dataset which also contained 4,096 spectra from an image of an uncontaminated latent fingerprint. These techniques also successfully and individually detected aspirin, diazepam and caffeine that had been deposited together in another fingerprint. Furthermore, they did not indicate any of these substances as a match in a search for another substance which was known not to be present. SAM was utilised to successfully locate explosive components in fingerprints deposited on silicon windows. The potential of other spectral searching algorithms employed in the field of remote sensing is considered. In addition, the applicability of the procedures tested in this work to other modes of spectral imaging is discussed

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J Anal Toxicol 2009 **33** (7) 372

Determination of digoxin and digitoxin in whole blood

The determination of digoxin and digitoxin in whole blood samples in autopsy cases was achieved with a liquid chromatography-tandem mass spectrometry (LC-MS-MS) procedure which has been both developed and validated. Samples were prepared by liquid-liquid extraction (LLE) with ethyl acetate/heptane/dichloromethane (3:1:1). LC separation was accomplished with an Atlantis dC_{18}-column (2.1 x 50 mm, 3 µm). The period between injections was 11 min. Mass detection was achieved with positive ion mode electrospray LC-MS-MS of the ammonium adducts with two transitions for each analyte and one for the internal standard (digoxin-d_3). Within-day precision was between 8.3 and 10.8%, between-day precision was between 8.7 and 14.2% and accuracy (bias) was between -17.3 and 11.5%. LOQ was 0.1 nmol/l (0.08 ng/ml), with an accuracy and precision of 19% and -17% (digoxin) and 18% and 3% (digitoxin). Matrix effects ranged from 104 to 117%. Satisfactory qualitative correlation was achieved for 38 autopsy cases when compared with previous findings. The median (range, number of cases) B-digitoxin and B-digoxin found with the LC-MS-MS protocol in this small scale study were 9.3 (3.4-23.8, n = 24) and 5.6 (3.4-26.5, n = 4) nmol/l, respectively

13 Alcohol

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Forensic Sci Int 2009 **189** (1-3) e45

Unreliable alcohol testing in a shipping safety programme
A case of an unphysiological urine ethanol concentration (235 mmol/l, 10.8 g/l) was discovered during a maritime alcohol and drug testing programme. The sample contained low levels of the ethanol metabolites ethyl glucuronide (EtG) and ethyl sulphate (EtS) which confirmed prior alcohol consumption. However, it also tested positive for the fermenting yeast *Candida albicans* which suggested the possibility of post-sampling ethanol formation. This and other questionable cases prompted an investigation of the suitability of urine alcohol testing for the intended application. In addition to the routine measurements of ethanol, illicit drugs and creatinine, randomly selected ethanol-positive and ethanol-negative urines collected within the maritime programme were analysed for the presence of EtG and EtS and for fungal and bacterial growth. Data on sample handling and storage was also collected. Ten of 15 (67%) ethanol-positive and 4 of 9 (44%) ethanol-negative urines contained yeast and/or bacteria. Of the ethanol-positive cases, 4 (27%) were clearly false positives because EtG and EtS were not detected. Microbial action as the cause of false-high ethanol concentrations was indicated in other cases. When 17 bacteria-infected but fungi-negative urines were supplemented with glucose and stored for 1 week at 21°C, ethanol was produced in 2 specimens containing *Escherichia coli* and *E. coli* plus *Pseudomonas aeruginosa*. In these samples, EtG was also formed on storage while EtS was not. The protocols employed for urine collection and handling within this substance abuse programme resulted in many false-positive identifications of alcohol use with unintended medico-legal consequences. Unpreserved urines stored without cooling should not be employed for alcohol analysis, given the high risk for microbial metabolic contamination

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Clin Chem 2009 **55** (7) 1395
Molecular species of the alcohol biomarker phosphatidylethanol in human blood measured by LC-MS
A group of ethanol-derived phospholipids formed from phosphatidylcholine by phospholipase D includes the alcohol biomarker phosphatidylethanol (PEth). PEth compounds have a common phosphoethanol head group connected to 2 fatty acids. An electrospray ionization (ESI) LC-MS technique for qualitative and quantitative measurement of different PEth species in human blood is described. A total lipid extract of whole blood was separated on an HPLC C4 column and LC-ESI-MS analysis was achieved with selected ion monitoring of deprotonated molecules for the PEth species and phosphatidylpropanol (internal standard). Identification of individual PEth species was based on ESI-tandem mass spectrometry (MS/MS) analysis of product ions. The major product ions of PEth were fatty acid moieties based on comparison with PEth-16:0/16:0, 18:1/18:1, and 16:0/18:1 reference material. For LC-MS analysis of different PEth species in blood, a calibration curve covering 0.2-7.0 µmol/l PEth-16:0/18:1 was employed. The lower limit of quantitation of the method was <0.1 µmol/l, and intra- and interassay CVs were <9% and <11%. In blood samples collected from 38 alcohol patients, the total PEth concentration ranges were from 0.1 to 21.7 µmol/l (mean 8.9). PEth-16:0/18:1 and 16:0/18:2 were the predominant molecular species, accounting for approximately 37% and 25%, respectively, of total PEth. PEth-16:0/20:4 and mixtures of 18:1/18:1 plus 18:0/18:2 (not separated using selected ion monitoring because of identical molecular masses) and 16:0/20:3 plus 18:1/18.2 made up approximately 13%, 12%, and 8%. The described LC-MS method facilitates simultaneous qualitative and quantitative measurement of several PEth molecular species in whole blood samples

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J Sep Sci 2009 **32** (20) 3497
Determination of ethyl glucuronide in human serum by capillary zone electrophoresis and an immunoassay
Ethyl glucuronide (EtG) is a marker of recent alcohol consumption. For the optimization of the analysis of EtG by CZE with indirect absorbance detection, the use of capillaries with permanent and dynamic wall coatings, the composition of the BGE, and various sample preparation procedures, including dilution with water, ultrafiltration, protein precipitation, and SPE, were investigated. Two validated screening assays for the determination of EtG in human serum, a CZE-based approach and an enzyme immunoassay (EIA), are described. The CZE assay uses a coated capillary, 2,4-dimethylglutaric acid as an internal standard, and a pH 4.65 BGE comprising 9 mM nicotinic acid, ε-aminocaproic acid and 10% v/v ACN. Proteins are removed *via* precipitation with ACN prior to analysis and the LOQ is 0.50 mg/l. The EIA is based upon commercial reagents which are promoted for the determination of urinary EtG. Krebs-Ringer solution containing 5% BSA is used as a calibration matrix. All samples are ultrafiltered prior to analysis of the ultrafiltrate on a Mira Plus analyzer. Assay calibration ranged between 0 and 2 mg/l and the upper reference limit was determined to be 0.05 mg/l. Both assays proved to be suitable for the analysis of samples from different individuals. For EtG levels above 0.50 mg/l, good agreement was observed for the comparison of the results of the two methods.

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J Chromatogr B 2009 **877** (23) 2337
Development and validation of a gas chromatography-negative chemical ionization tandem mass spectrometry method for the determination of ethyl glucuronide in hair and its application to forensic toxicology
A minor and direct metabolite of ethanol is ethyl glucuronide (EtG). EtG is incorporated into the growing hair thus facilitating retrospective investigation of chronic alcohol abuse. The development and the validation of a technique employing gas chromatography-negative chemical ionization tandem mass spectrometry (GC-NCI-MS/MS) for the quantification of EtG in hair is described. EtG was extracted from about 30 mg of hair by aqueous incubation. It was purified by solid-phase extraction (SPE) utilising mixed mode extraction cartridges and subsequent derivation with perfluoropentanoic anhydride (PFPA). The analysis was carried out in the selected reaction monitoring (SRM) mode emplying the transitions *m/z* 347→163 (for the quantification) and *m/z* 347→119 (for the identification) for EtG, and *m/z* 352→163 for EtG-d_5 emplyed as internal standard. For validation, quality controls (QC) were prepared using hair samples taken post mortem from 2 subjects with a known history of alcoholism. Samples were confirmed by proficiency analyses by 7 participating laboratories. The assay linearity of EtG was confirmed over the range from 8.4 to 259.4 pg/mg hair, with a coefficient of determination (r^2) above 0.999. The limit of detection (LOD) was estimated with 3.0 pg/mg. The lower limit of quantification (LLOQ) of the technique was fixed at 8.4 pg/mg. Repeatability and intermediate precision (relative standard deviation, RSD%), tested at 4 QC levels, were less than 13.2%. The analytical procedure was employed with several hair samples obtained from autopsy cases with a history of alcoholism and/or lesions caused by alcohol. EtG concentrations in hair ranged from 60 to 820 pg/mg hair

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J Anal Toxicol 2009 **33** (6) 328
Long-term stability of ethanol solutions for breath-alcohol tests
Water/ethanol solutions in conjunction with a simulator are usually employed to calibrate and check the operation of breath-alcohol sensors. In Ohio, the reference solutions have an expiry one year from the date of manufacture. This study was to investigate the stability of several batches of solution that had been produced between 2003 to 2007 and were classified as statutorily expired. Eight batches of solution, each with a theoretical breath-alcohol target value of 0.100 g/210 liter were analysed on paired Intoxilyzer® 8000s using Guth model 590 simulators. This model of simulator required only 250 ml of solution each. Each batch was evaluated by analysis of a single bottle of solution that had remained sealed since its production. Five tests were performed on each instrument and the acceptable criterion was limited to +/- 0.005 g/210 liter of the established target value. A current batch of solution was also analysed to validate the performance of the instrument both before study sample testing commenced and following completion of testing. All batches and bottles produced satisfactory results within the acceptable limit for each target value. It was deduced that the stability of the alcohol solution packaged in high-density polyethylene bottles with a theoretical concentration of 0.100 g/210 liter is in excess of five years. In addition, it exceeds the statutory expiration term of one year from the date of production when stored at normal room temperatures

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Electrophoresis 2009 **30** (12) 2207
A proteomic workflow for discovery of serum carrier protein-bound biomarker candidates of alcohol abuse using LC-MS/MS
A dearth of sensitive and specific screening and monitoring tests is hampering the diagnosis and care of patients with alcohol abuse and dependence. Proteomics is a increasingly valuable approach to search for biomarkers including those of alcohol abuse. Serum carrier protein-bound proteins have attracted significant interest because they remain a relatively little investigated region of the proteome. The discovery of novel biomarker candidates of alcohol abuse was investigated with a proteomic workflow including LC-MS/MS with enrichment of serum carrier protein-bound biomarkers in order to profile the changes in quality and quantity of serum carrier protein-bound proteins. A total of 311 proteins were identified with high confidence were noted to be bound to serum carrier proteins. Complement isoforms, Ig fragments, and apolipoprotein family proteins are the main serum

carrier-bound proteins. Gender was found to be a critical consideration for biomarker development of alcohol abuse. Proteins not previously associated with alcohol abuse included gelsolin, selenoprotein P, serotransferrin, tetranectin, hemopexin, histidine-rich glycoprotein, plasma kallikrein, and vitronectin. Altered amounts of these proteins indicates that they may be potential novel biomarkers for alcohol abuse

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Proteomics Clin Appl 2009 **3** (7) 821

A search for novel markers of alcohol abuse using magnetic beads and MALDI-TOF/TOF mass spectrometry

Objective markers are required to assess excessive alcohol consumption, which can lead to a various medical and social problems. In this study, we carried out serum peptidome analyses using the ClinProt™ system, which consists of magnetic beads and MALDI-TOF/TOF MS, to find novel biomarkers of alcohol abuse in 16 chronic alcoholic patients that were hospitalized for a rehabilitation program. A total of 22 peaks were found to be significantly altered during abstinence. Out of these 22 peaks, 3 peaks that had an *m/z* of 3000 or less and substantial peak intensities were subjected to MS/MS analysis followed by a MASCOT search. The 1466 Da and the 1616 Da peptides were upregulated on admission and were identified as fragments of fibrinopeptide A and phosphorylated fibrinopeptide A, respectively. On the other hand, the 2660 Da peptide, which was downregulated on admission and increased during abstinence, was identified as a fragment of the fibrinogen a C chain. These peaks were not detectable by the SELDI-TOF MS ProteinChip® system analysis. The alterations in these peaks induced by alcohol abuse were also seen in y glutamyltransferase nonresponders. These protein fragments may be additional biomarkers for excessive alcohol drinking

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Addiction 2009 **104** (12) 2007

Urine tested positive for ethyl glucuronide after trace amounts of ethanol

Ethyl glucuronide (EtG) is used commonly as a marker for the detection of non-compliance of patients in alcohol withdrawal therapy in psychiatric hospitals in Europe and in work-place monitoring programmes in the United States. With the increased use of this new marker, questions related to an unintentional uptake of ethanol resulting in detectable EtG concentrations have been discussed. The aim of this study was to determine the concentration ranges of EtG and ethyl sulphate (EtS) after the consumption of very small amounts of ethanol (1 and 3 g), which are more likely to be incidental than intended. Drinking experiments with ethanol amounts of 1 and 3 g, respectively, were performed on a total of 31 volunteers. EtG and EtS analysis in urine was performed by electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), and creatinine concentration was determined using the Jaffé reaction. Furthermore, data obtained from this experimentation were then compared to data from literature. The maximum concentration of EtG normalized to creatinine after the uptake of 1 g and 3 g of ethanol was found to be 0.32 mg/l and 1.53 mg/l, respectively, and 0.15 mg/l and 1.17 mg/l for EtS; these peak concentrations are considered to be positive by many laboratories testing urine for ethanol conjugates in work-place testing progammes.

15 Homeland Security

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Biosens Bioelectron 2009 **25** (2) 469

Square wave voltammetric detection of anthrax utilizing a peptide for selective recognition of a protein biomarker

The diagnosis of anthrax has been achieved by utilising a short chain peptide (16mer) for the selective electrochemical detection of the protein biomarker, protective antigen (PA). The primary consideration for employing a peptide rather than an antibody in the production of a biosensor is that there are benefits associated with smaller size, better biological stability and easier synthesis. PA-selective peptide was synthesized and conjugated onto a binding layer previously immobilized on gold electrode. An identical scheme but using a conventional antibody instead of a peptide was also tested for the purpose of comparison. The size of the peptide is approximately 1-2 orders of magnitude smaller than that of the antibody. Therefore, a more populated immobilization of peptide on the sensing layer is possible and this should result in improved sensitivity for PA detection. The selectivity of the peptide-based sensor was tested by noting the reduction in peaks of samples containing PA containing different concentrations of bovine serum albumin (BSA). Responses proved to be interference-free with respect to BSA. Results of this research illustrate the robust analytical possibilities of a peptide-based biosensor for diverse applications. This may be of particular interest in disease diagnosis where detection of a specific protein biomarker is particularly demanding

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Chromatographia 2009 **70** (3-4) 623

Solid supported liquid-liquid extraction of chemical warfare agents and related chemicals from water

Solid-supported liquid-liquid extraction was optimized to extract the chemical warfare agents and their non-toxic analogues from water. The developed method was compared to the conventionally used liquid-liquid extraction. This method yielded high recoveries (70-80%) of non-toxic analogues of chemical warfare agents and good recoveries (65-75%) of the nerve agent sarin and Lewisite-III. The limits of detection of non-toxic analogues of CWAs, and toxic sarin and Lewisite-III, in selected ion monitoring and full scan mode, varied from 0.01 to 0.5 μg/ml and 0.1 to 1.0 μg/ml respectively

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Anal Sci 2009 **25** (7) 845

Development of spray deposition/MALDI-TOFMS and its application to the rapid screening of hydrolysis products derived from nitrogen mustards

A new method for preparing samples for use in MALDI-TOFMS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) has been developed. Seven hydrolysis products produced from nitrogen mustards were selected as model compounds and CHCA (α-cyano-4-hydroxycinnamic acid) and was employed as the matrix. A capillary atomizer was employed for evaporative and spray deposition of the sample/matrix solution,resulting in the formation of a freestanding film which coated and accumulated on the MALDI substrate (i.e., the sample plate). Compared with the usual method for MALDI, which involves the production of dried droplets, the surface roughness was reduced, resulting in the accumulation of the sample-doped matrix on the sample plate. This produced an increase in the limit of detection of 1 - 2 orders of magnitude. Scanning electron microscopy (SEM) was employed to compare the structures of the sample-doped matrices obtained by the traditional dried droplet method *versus* the spray deposition method (developed in this study). The design of the capillary atomizer and details of the experimental conditions are described. The application of this method to the hydolysis products was successful, suggesting that it has great potential for use as a routine monitoring tool

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Anal Chem 2009 **81** (16) 6744

Quantitation of chemical warfare agents using the Direct Analysis in Real Time (DART) techniques

Direct Analysis in Real Time (DART) is an ion source that facilitates rapid mass spectrometric analysis of gases, liquids, and solids in open air under ambient conditions. Within the field of chemical weapons sensors it provides a unique technology which does not require a vapor pressure, does not require sample preparation, and is nondestructive to the original sample. The DART procedure has had success as a first line instrument for detection of chemical warfare agents. However, there have been remaining questions in respect of the technique's quantitative reliability and reproducibility. Herein its capability to produce linear calibration curves (r^2 = 0.99 or better) for the nerve agents GA, GB, and VX as well as the blister agent HD is demonstrated. Independently produced standards measured against these curves typically have recovery errors less than 3%. The DART instrument response is established to be linear over roughly 3 orders of magnitude. Furthermore, this study illustrates that averaging as few as three measurements for each data point is adequate to produce high quality calibration curves. Therefore, data collection time is reduced and results provided faster

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J Agric Food Chem 2009 **57** (18) 8227

Quantitative analysis of chemical warfare agent degradation products in beverages by liquid chromatography tandem mass spectrometry

Chemical warfare agents (CWAs) have been banned by the Chemical Weapons Convention. However, the threat that such chemicals may be deployed, including their deliberate addition to food, endures. CWAs may hydrolyze to phosphonic acidsin such matrices and these are suitable surrogate markers of CWA contamination. A protocol is described for the extraction of five CWA degradation products, including methylphosphonic acid (MPA), ethyl-MPA,

isopropyl-MPA, cyclohexyl-MPA, and pinacolyl-MPA, from five different beverages by strata-X solid phase extraction cartridges. Samples were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) with multiple reaction monitoring. The limit of quantitation ranged from 0.05 to 0.5 ng on-column, and the limit of detection was >0.02 ng on-column. Beverages were spiked with the five phosphonic acids at 1 µg/ml and 0.25 µg/ml and quantitated with both an internally standardized method and matrix-matched standards. Acceptable recoveries (>50%) were accomplished for ethyl, isopropyl, cyclohexyl, and pinacolyl-MPA in most matrixes

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J Mass Spectrom 2009 **44** (11) 1604

Mass spectrometry as a tool for characterization of *N,N*-dialkylaminoethane-2-thiols-precursors and degradation products of chemical warfare agents

N,N-dialkylaminoethane-2-thiols belong to the group of precursors and degradation products of chemical warfare agents (CWAs). These compounds were analyzed by means of electrospray ionization-multiple stage mass spectrometry (ion trap) and proposed fragments were confirmed by accurate mass measurement using a QqTOF system. The fragmentation pathways of studied compounds and the products of oxidation (formation of -S-S- linkage) were described. Some minor interesting processes, such as rearrangement of SH group, were observed and proved. A new microLC/MS method, based on ion-pairing chromatography, was developed. Trifluoroacetic acid was employed as an ion-pairing agent to increase the low retention of compounds of interest in the reverse-phase system. The technique was compared with the UPLC/MS method, allowing fast analysis of all the studied thiols as well as an explorative control of originated disulfides

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Rapid Commun Mass Spectrom 2009 **23** (23) 3875

Real-time trace detection and identification of chemical warfare agent simulants using recent advances in proton transfer reaction time-of-flight mass spectrometry

This work demonstrates for the first time the potential of using recent developments in proton transfer reaction mass spectrometry for the rapid detection and identification of chemical warfare agents (CWAs) in real-time. A high-resolution (m/Δm up to 8000) and high-sensitivity (approximately 50 cps/ppbv) proton transfer reaction time-of-flight mass spectrometer (PTR-TOF 8000 from Ionicon Analytik GmBH) has been successfully used to detect a number of chemical warfare agent simulants at room temperature; namely dimethyl methylphosphonate, diethyl methylphosphonate, diisopropyl methylphosphonate, dipropylene glycol monomethyl ether and 2-chloroethyl ethyl sulfide. Importantly, we demonstrate in this paper the potential to identify CWAs with a high level of confidence in complex chemical environments, where multiple threat agents and interferents could also be present in trace amounts, thereby reducing the risk of false positives. Instantaneous detection and identification of trace quantities of chemical threats using proton transfer reaction mass spectrometry could form the basis for a timely warning system capability with greater precision and accuracy than is currently provided by existing analytical technologies

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Clin Toxicol 2009 **47** (7) 643

Broad recognition of ricin toxins prepared from a range of *Ricinus* cultivars using immunochromatographic tests

It is vital that diagnosis of persons exposed to ricin toxin be accomplished as quickly as possible for triage and appropriate treatment. Recently, a sensitive and specific immunochromatographic test (ICT) for ricin has been produced. The ICT detected ricin of a single cultivar, by naked eye, at concentrations as low as 1 ng/ml. However, *Ricinus communis* exists as many cultivars and each produces ricin. In addition, significant differences in ricin toxicity between two cultivars have been previously established. Ricins extracted from different cultivars could demonstrate variations in primary sequence or glycosylation resulting in differences in the limits of detectability. The ICT was employed with solutions of ricin isolated from a wide range of cultivars to determine whether it could be detected in all instances. Also investigated was whether there were differences between the limits of visible detection of ricin and also in the amounts using a densitometer. The ICT successfully recognised ricins isolated from 19 *Ricinus* cultivars and all with a limit of visible detection between 1 and 2.5 ng/ml in buffer. However, this was reliant to some extent on whether the operator was experienced or not in reading the test. This rapid and sensitive test provides a method for the rapid diagnosis of ricin poisoning. It will now be evaluated in animal models following inhalational exposure to ricin. This sensitive and rapid technique may provide a timely diagnosis to inform the deployment of a ricin antitoxin currently under development at Defence Science and Technology Laboratory in the United Kingdom

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J Chromatogr A 2009 **1216** (31) 5911

Determination of nerve agent degradation products by capillary electrophoresis using field-amplified sample stacking injection with the electroosmotic flow pump and contactless conductivity detection

Four nerve agent degradation products methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA) and cyclohexyl methylphosphonic acid (CMPA) were analysed by employing field-amplified sample stacking injection with an electroosmotic flow pump (FAEP) for capillary electrophoretic separation. When coupled to contactless conductivity detection it resulted in direct quantification of these non-UV active compounds. Sensitivity enhancement of up to 500 to 750-fold could be achieved. The developed technique was applied to the analysis of river water and aqueous extracts of soil. Detection limits of 0.5, 0.7, 1.4 and 2.7 ng/ml were produced for MPA, EMPA, IMPA and CMPA, respectively, in river water and 0.09, 0.14, 0.44 and 0.22 µg/g, respectively, in soil

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Int J Environ Anal Chem 2009 **89** (8-12) 821

Detection of ricin toxin in water using immunoassays

Two types of immunoassays were utilised to determine ricin toxin through the substitute of A chain sub-unit in water. A prodeure for detecting ricin by commercial enzyme-linked immunosorbent assay (ELISA) kits was developed and validated. Final results were produced by this assay in six hours and the limit of detection was found to be less than 3 ng/ml based on ricin A chain. The was minimal cross-reactivity from ricin B chain. The validated assay was successfully employed in the determination of ricin toxin in water for the purpose of water security. No evidence of this biotoxinwas found in water samples. In order to decrease the assay time and enhance the ability of securing water quality, a fibre optic biosensor RAPTOR™ was applied as another platform to rapidly analyse ricin toxin in water. Polyclonal anti-ricin antibody was captured on the waveguide through incubation. Four waveguides were assembled into each coupon, which could facilitate multiplex analysis in one assay run. Cy5-labelled antibody was employed as detector antibody and characterised by MALDI-TOF mass spectrometry. Results indicated that the detection limit was 10 and 60 ng/ml of ricin A chain in deionised water and tap water, respectively. Based on readily assembled coupon, the assay run could be completed and the results reported in less than 15 minutes. Therefore, the biosensor has been shown to be a promising technique for rapid and sensitive detection of biotoxins in water. Future studies will aim to further improve the sensitivity and enhance reproducibility of the assay for the detection of ricin toxin.

16 Workplace

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Anal Bioanal Chem 2009 **395** (4) 1167

Quantification of two urinary metabolites of organophosphorus flame retardants by solid-phase extraction and gas chromatography-tandem mass spectrometry

Flame retardants commonly contain trialkyl esters of phosphoric acid. Corresponding dialkylphosphates are formed as the primary metabolites in animal experiments. A previously published procedure for the determination of four organophosphorus flame retardant metabolites [bis(2-chloroethyl) phosphate, diphenyl phosphate, di-*m*-cresyl phosphate and di-*p*-cresyl phosphate] has been developed to be able to determine di-*n*-butyl phosphate (DBP) and bis(2-chloropropyl) phosphate (BCPP) in human urine samples additionally in one run. Following solid-phase extraction, derivatization with pentafluorobenzyl bromide and further solid-phase cleanup, the extracts were analysed by gas chromatography-tandem mass spectrometry. The limits of detection were 0.25 µg/l for both di-*n*-butyl phosphate (DBP) and bis(2-chloropropyl) phosphate (BCPP). Interday imprecisions were 2-6%. To validate the technique, the internal burden of 25 persons of the population was determined. Twelve percent of the urine samples tested positive for BCPP at concentrations from below the limit of detection to 0.85 µg/l and one sample contained 0.26 µg/l DBP

17 Product Authenticity

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J Pharm Biomed Anal 2009 **50** (4) 602

2D and 3D DOSY ^{1}H NMR, a useful tool for analysis of complex mixtures: Application to herbal drugs or dietary supplements for erectile dysfunction

Diffusion ordered spectroscopy (DOSY) ^{1}H NMR was employed to analyse seventeen herbal dietary supplements, marketed as natural substances for the enhancement of sexual function. The procedure facilitated global analysis of the samples with determination of both active and inactive ingredients present in these complex matrixes. Compounds related to the synthetic phosphodiesterase-5 inhibitors were discovered in eight formulations. Sildenafil, tadalafil, vardenafil, hydroxyhomosildenafil, thiosildenafil, and the newly identified adulterant thiomethisosildenafil were identified. Quantification of these active ingredients was achieved with HPLC or NMR. In addition to these active compounds, approximately 30 compounds or excipients were characterized. Three-dimensional DOSY-COSY ^{1}H NMR was utilised to analyse a herbal formulation and provided both virtual separation and structural information

18 Techniques

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Biosens Bioelectron 2009 **25** (2) 502

Strip-based immunochromatographic assay using specific egg yolk antibodies for rapid detection of morphine in urine samples

Rapid urinalysis of morphine was achieved using specific egg yolk antibodies (IgY) in a strip-based immunochromatographic assay. The IgY type antibody against morphine was produced by immunizing chickens with well-characterized monoacetyl morphine-protein conjugate. The antibody was labeled with gold nanoparticles and employed as an immunoprobe in the dipstick format for the visual detection of morphine in urine samples. The dipstick was produced by utilising three membranes. Firstly, an application pad made of glass fiber membrane to hold the tracer. Secondly, a signal generation test line on nitrocellulose membrane (detection zone). Finally, a cellulose membrane used as an absorption pad. Morphine and its analogues were added to the sample well, dissolved the labeled antibody (tracer), and the antigen-antibody complex formed was transported by the flow caused by capillary action to the test line. The color signal of test line was in proportion to the morphine concentration in urine samples and was measured using a detector. The developed dipstick assay format was optimized, showing the average IC_{50} values of morphine as low as 9.45 ng/ml, the detection range of 1-1000 ng/ml and the lowest detection limit 2.5 ng/ml under optimal conditions. The correlation between the developed dipstick and ELISA was 0.948 in the urinalysis for samples spiked with morphine. The developed dipstick could prove to be a highly sensitive and convenient method for the rapid detection of opiate drugs in samples with a high degree of stability

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Forensic Sci Int 2009 **189** (1-3) 14

Use of stable isotope ratios for profiling of industrial ephedrine samples: Application of hydrogen isotope ratios in combination with carbon and nitrogen

An evaluation was made of the efficacy of hydrogen stable isotope ratio measurement by IR-MS for confirming the origin of ephedrine and pseudoephedrine (ephedrines), precursors of methamphetamine. There are two types of commercial semisynthetic ephedrines, one produced from molasses and the other from pyruvic acid. Whereas semisynthetic ephedrines produced from pyruvic acid cannot be differentiated from biosynthetic ephedrines and synthetic ephedrines based on δ^{13}C and δ^{15}N values, they could be identified from the δ^{2}H values. The low deuterium content of biosynthetic ephedrines (δ^{2}H: -193 to -151 per thousand) allows a clear distinction from synthetic ephedrines (δ^{2}H: -73 to -30 per thousand), semisynthetic ephedrines derived from pyruvic acid (δ^{2}H: +75 to +148 per thousand) and semisynthetic ephedrines derived from molasses (δ^{2}H: -74 to +243 per thousand). Therefore, the wide range of δ^{2}H values of semisynthetic ephedrines facilitates the detailed classification of ephedrines, in combination with the measurement of δ^{13}C and δ^{15}N values as described previously. This investigation was conducted on a limited number of samples but which reflect the various routes of ephedrines manufacture. It has become clear that stable-isotope analysis is an useful means by which to screen for the manufacturing process of ephedrines. This technique could be important for worldwide precursor control of methamphetamine

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Interpretive optimisation of organic solvent content and flow-rate in the separation of -blockers with a chromolith RP-18e column

The chromatographic performance of a Chromolith RP-18e column was comprehensively examined for a group of basic drugs (β-blockers), eluted with isocratic ACN-water mixtures at increasing flow-rate up to 6 ml/min. As the flow-rate increases at fixed mobile phase composition, peak distribution (selectivity) is maintained, but the relative peak widths increase. This reduces the resolution below satisfactory values for closely eluting compounds. With the monolithic column, flow-rate becomes thus an important factor to be optimised, in addition to the mobile phase composition. Since, theoretically, retention factors (k) are independent of the flow-rate, the classical quadratic model relating log k with the solvent content allows the prediction of the retention at any combination of organic solvent content and flow-rate. The small deviations found for the most retained compounds were corrected by including, in the quadratic model, an additional term correlating linearly log k with the flow-rate. Peak shape and resolution changes were predicted by taking advantage of the approximated linear relationships between peak half-widths and retention times, which offered similar coefficients for peaks eluting at different organic solvent contents and flow-rates. The accuracy of the predictions in critical conditions was experimentally verified to be satisfactory

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J Sep Sci 2009 **32** (15-16) 2841

Performance of a chromolith RP-18e column for the screening of -blockers

The chromatographic performance of a monolithic column (Chromolith RP-18e) was comprehensively examined in the isocratic separation of ten β-blockers, using ACN-water mobile phases, and compared with the performance of three microparticulate RP columns manufactured with different types of silica: Spherisorb ODS-2, Kromasil C18 and XTerra MS C18. The comparison considered the analysis time, selectivity, peak shape (column efficiency and asymmetry) and resolution, and was extended to a wide range of mobile phase compositions. The Chromolith column showed good performance for the analysis of β-blockers with regard to the packed columns. In terms of selectivity and analysis time, the greatest similarity was found between the Chromolith and XTerra columns. The addition of a silanol blocking agent (0.1% triethylamine) to both Chromolith and Spherisorb columns yielded, apparently, a similar blocking degree of the silanol groups (based on the similar peak shapes), and gave rise to similar selectivity

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RP-LC simultaneous determination of aconitine, solanine and piperine in an ayurvedic medicine

The simultaneous quantitative determination of aconitine, solanine and piperine in an ayurvedic preparation prepared from *Aconitum ferox*, *Solanum indicum*, *Piper nigrum* and *Piper longumhas* been achieved with a reliable, rapid, simple and accurate liquid chromatography method with UV detection following optimization of the extraction, separation and analytical parameters. The separation of these alkaloids was achieved on an reversed phase C_{18} column (250 mm x 4.6 mm ID, 5 μm particle size), with isocratic elution using a mixture of acetonitrile potassium hydrogen phosphate buffer (10 mM, pH 7.5)-methanol (60:25:15, v/v) at a flow rate of 1 ml/min with UV detection at 227 nm for aconitine and solanine while 343 nm for piperine. The calibration curves were linear with correlation coefficients of 0.9990, 0.9942, 0.9989 for solanine, piperine and aconitine, respectively. The % relative standard deviation (%RSD) values were less than 2% in the concentration range of 10-100 μg/ml for all the three alkaloids. Intra-day assay and inter-day assay precision of the analytes were less than 2%, and the average recovery rates obtained were in the range of 98-102% for all with %RSD below 2%. The marketed formulations exhibited significant variations in alkaloids. The developed method furnish manufacturers with the basis of a quality control programme. In addition, it could provide the public with quality and safety assurance of the proprietary ayurvedic formulations