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Current awareness in drug testing and analysis

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1 Reviews

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Forensic Sci Int 2009 **185** (1-3) 1
Analysis of toxic alkaloids in body samples
Toxic alkaloids, be dangerous to humans may be found in many plants. However, cases of fatal plant poisonings are relatively rare considering the large number of poisonous plants. The plants involved are often regionally specific and consequently, the frequencies of poisonings. Three categories of plant poisonings may be identified as unintended ingestions, intended ingestions, and poisoning due to abuse of plant material. Unintended ingestions frequently involve children. Inddition, misidentification of plants and mushrooms may result in poisoning in adults. Intended ingestions are a feature of homicides and suicides where poisonous plants are involved. The abuse of plants for hallucinogenic reasons is becoming increasingly common. The diagnosis of poisoning or abuse cases may be facilitated by oxicological analysis of such alkaloids. This review describes the toxic alkaloids aconitine, atropine, coniine, colchicine, cytisine, dimethyltryptamine, harmine, harmaline, ibogaine, kawain, mescaline, scopolamine, and taxine, frequently involved in fatal and non-fatal poisonings. In addition, the paper summarizes the symptoms of the intoxications and reviews techniques for detection of their toxic constituents in biological fluids

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Semin Diagn Pathol 2009 **26** (1) 18
A review of the use of ethyl glucuronide as a marker for ethanol consumption in forensic and clinical medicine
The UDP-glucuronosyl transferase catalyzed conjugation of ethanol with glucuronic acid results in ethyl glucuronide (EtG). A variety of analytical methods has identified EtG in many antemortem and postmortem biological matrices. The use of EtG has been proposed as a marker of recent ethanol intake in a variety of clinical and legal settings, including medical monitoring for relapse, emergency department patient evaluation, postmortem assessments, and transportation accident investigation due to its long urinary elimination time, detectability in hair, specificity for ethanol exposure, and low detection limits of assays. Accurate interpretation of results is substantially complicated by challenges associated with factors such as establishing appropriate cut-off levels capable of distinguishing between drinking and nonbeverage sources of ethanol exposure, nonuniform laboratory reporting limits, sample stability, and microbial activity. This paper briefly reviews the history, utility, and limitations of EtG in contemporary medical and forensic practice

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Steroids 2009 **74** (3) 288
Steroid analysis and doping control 1960-1980: Scientific developments and personal anecdotes
Before the introduction of combined gas chromatography/mass spectrometry (GC/MS), definitive proof of anabolic steroid abuse in sports was not possible. This paper summarises the early history (1960-1980) of GC/MS and radioimmunoassay, and how these techniques were employed in the first years of steroid doping control in athletics. There were several key individuals and research groups involved in the early technical developments, and their essential contributions have been acknowledged. Our laboratory was the first IAAF (International Association of Athletic Federations) sanctioned site to do steroid GC/MS steroid analysis resulting in athletes being disqualified from competition. There were notable successes for example, the only East German female competitor ever suspended during the tenure of the Deutsche Demokratische Republik. In addition to recording the scientific advances and milestones in the incorporation of steroid testing into international athletics, the paper includes personal anecdotes of the early years before doping control became justifiably regimented. By the early 1980s and in readiness for the Los Angeles Olympic games, dedicated year-round sports testing facilities had been established

Singh S, Prasad B, Savaliya AA, Shah RP, Gohil VM, Kaur A// NIPER, Dept Pharmaceut Anal, Sector 67, IN-160062 Sas Nagar, India
Trends Anal Chem 2009 **28** (1) 13
Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs
There is widespread use of synthetic phosphodiesterase type-5 (PDE-5)-inhibitor drugs (*viz* sildenafil, vardenafil and tadalafil), which are constituents of popular brands (*viz* Viagra, Levitra and Cialis, respectively) as adulterants in herbal dietary supplements (HDSs) for the treatment of erectile dysfunction in males. In addition, their unapproved analogues have been detected in HDSs. Identifying them is becoming more problematic as concealed, structurally modified analogues are increasingly being used. Moreover, counterfeits of the popular brands have appeared. However, it has become possible to detect these drugs and their derivatives as adulterants and counterfeits by employing modern sensitive and selective analytical techniques [e.g., liquid chromatography with tandem mass spectrometry, Fourier transform (FT) with near infrared spectrometry, and FT with Raman spectroscopy]. The literature is critically reviewed and generalized strategies presented, including flow charts, for characterizing adulteration of PDE-5 inhibitors in HDSs, and detecting and categorizing counterfeit products

In order to keep subscribers up-to-date with the latest developments in their field, John Wiley & Sons are providing a current awareness service in each issue of the journal. The bibliography contains newly published material in the field of drug testing and analysis. Each bibliography is divided into 18 sections: 1 Reviews; 2 Sports Doping - General; 3 Steroids; 4 Peptides; 5 Diuretics; 6 CNS Agents; 7 Equine; 8 Recreational Drugs - General; 9 Stimulants; 10 Hallucinogens; 11 Narcotics; 12 Forensics; 13 Alcohol; 14 Tobacco; 15 Homeland Security; 16 Workplace; 17 Product Authenticity; 18 Techniques. Within each section, articles are listed in alphabetical order with respect to author. If, in the preceding period, no publications are located relevant to any one of these headings, that section will be omitted.

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3 Steroids

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Steroids 2009 **74** (3) 393

Isotope ratio mass spectrometry analysis of the oxidation products of the main and minor metabolites of hydrocortisone and cortisone for anti-doping controls

HPLC fractionation of urine extracts was employed to separate metabolites of hydrocortisone (HC) and cortisone (C), namely tetrahydrocortisol (THF), tetrahydrocortisone (THE), allo-THF, allo-THE for the main metabolites and 11-hydroxyandrosterone, 11-hydoxyetiocholanolone, 11-ketoandrosterone, and 11-ketoetiocholanolone for the minor metabolites, as well as the two main metabolites of testosterone, androsterone and etiocholanolone. Isotopic ratio mass spectrometry (IRMS) was used to produce the absolute $\delta^{13}C$ values of 5α-androstanetrione (5α-AT) and 5β-androstanetrione (5β-AT) as the oxidation products (ox-products) of the HC and C metabolites and as target compounds (TCs). IRMS analysis of 5α-androstanedione (5α-AD) and 5β-androstanedione (5β-AD) was also employed for the ox-products of etiocholanolone and androsterone and as endogenous reference compounds (ERCs). Urine specimens were taken from two male volunteers treated with a single 10-mg oral dose and a single 100-mg intramuscular dose of HC hemisuccinate, a male volunteer treated with a single 25-mg oral dose of C acetate, and a control group of 30 drug-free athletes. The mean -3SD of $\delta^{13}C$ depletion values from the controls were -1.46, -1.98, -1.78 and -2.42 for 5β-AT-5β-AD, 5α-AT-5β-AD, 5β-AT-5α-AD and 5α-AT-5α-AD, respectively, indicating -3 per thousand as a safe cut-off value for differentiating the pharmaceutical from the natural form. Analysis of the main metabolite fraction, $\delta^{13}C$ depletion values peaked around -5 per thousand and -9 per thousand after oral and intramuscular administration of HC, respectively, and around -6 per thousand after oral administration of C. However, less striking results were produced when IRMS analysis focused on the ox-products of the minor metabolites

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Steroids 2009 **74** (3) 379

Carbon isotope ratio ^{13}C values of urinary steroids for doping control in sport

A significant challenge for doping control laboratories accredited by the World Anti-Doping Agency (WADA) is the detection of steroids originating from synthetic precursors compared with their chemically identical natural analogues. The precise measurement of differences in stable isotope ratios that arise as a result of fractionation patterns inherent in the source of steroids facilitates the detection of endogenous steroid abuse utilising the atomic specificity of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). A comprehensive carbon isotope ratio ($\delta^{13}C$) profiling study (n=1262) of urinary ketosteroids has been produced which illustrates the inter-individual variation that may be expected from factors such as nutrition, ethnicity, gender and age within and between different populations (13 countries). The $\delta^{13}C$ distribution obtained by principal component analysis (PCA) provides a statistical comparison of $\delta^{13}C$ values produced following administration of testosterone enanthate. A limited collection of steroid diol data (n=100; consisting of three countries) was also produced with comparison to $\delta^{13}C$ values of excreted testosterone to validate criteria for WADA accredited laboratories to prove doping offences

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Steroids 2009 **74** (3) 296

Androgens and bone

The major gonadal sex steroid produced by the testes in men is testosterone. It is also produced in smaller amounts by the ovaries in women. The weaker androgens dehydroepiandrosterone, dehydroepiandrosterone sulfate, and androstenedioneare produced by the adrenal glands. Together, these androgens affect lifelong skeletal homeostasis in both men and women, particularly at puberty and during adult life. Testosterone may be metabolized to estradiol by the aromatase enzyme and thus there has been controversy as to which gonadal sex steroid has the greater skeletal effect. Current evidence suggests indicates that estradiol plays the a greater role in maintenance of skeletal health but that androgens also have direct beneficial effects on bone. Supraphysiological levels of testosterone probably have similar effects on bone as lower levels through direct interaction with androgen receptors, as well as effects mediated by estrogen receptors after aromatization to estradiol. High doses of synthetic, non-aromatizable androgens may be detrimental to bone due to suppression of endogenous testosterone (and estrogen) levels and thus a potential concern that warrants further investigation

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Steroids 2009 **74** (3) 306

Identification of drostanalone and 17-methyldrostanolone metabolites produced by cyropreserved human hepatocytes

Methyldrostanolone (2α,17α-dimethyl-17β-hydroxy-5α-androstan-3-one) was synthesized from drostanolone (17β-hydroxy-2α-methyl-5α-androstan-3-one) and identified in commercial products. The biotransformation of drostanolone and its 17-methylated derivative was studied in cultures of cryopreserved human hepatocytes. The common 3α- (major) and 3β-reduced metabolites of both steroids were identified by GC-MS analysis of the extracted culture medium and the stereochemistry confirmed by incubation with 3α-hydroxysteroid dehydrogenase. Structures corresponding to hydroxylated metabolites in C-12 (minor) and C-16 were suggested for other metabolites based upon the analysis of the mass spectra of the pertrimethylsilyl (TMS-d_0 and TMS-d_9) derivatives. On the basis of the GC-MS and ^1H NMR data and through chemical synthesis of the 17-methylated model compounds, structures could be developed for metabolites hydroxylated in C-2. All the metabolites extracted from hepatocyte culture medium were present in urine collected following the administration to a human volunteer albeit in different relative amounts. This study affirms the suitability of the cryopreserved hepatocytes to produce characteristic metabolites and study biotransformation of new steroids

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Sensor Actuator B Chem 2009 **137** (2) 676

Sensitive voltammetric sensor for determination of flumethasone pivalate, abused for doping by athletes

The voltammetric reduction of flumethasone pivalate has been studied at fullerene-C_{60}-modified edge plane pyrolytic graphite electrode (PGE) and two well defined peaks were produced with a peak potential of ~-1220 mV and ~-1351 mV respectively. The modified electrode demonstrated a superior response when compared with a bare basal plane PGE and bare edge plane PGE. Linear calibration curves were produced with sensitivity of 0.685 μA μM and 0.570 μA/μM and the corresponding limits of detection at both the peaks have been found to be 13.81 x 10^{-8} M and 27.5 x 10^{-8} M. Recovery studies for flumethasone pivalate in biological samples were also conducted

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Steroids 2009 **74** (3) 350

Excretion of norsteroids' phase II metabolites of different origin in human

Samples collected during pregnancy, following the administration of norsteroids or the consumption of edible parts of non-castrated pig and in athletes' samples in which they were detected during routine controls were analysed for urinary phase II metabolites of norsteroids, 19-norandrosterone, 19-noretiocholanolone and 19-norepiandrosterone glucuronide and sulphate. Following selective hydrolysis, the sulfo- and glucuroconjugated metabolites were quantified by GC/HRMS. The object of the investigation was to determine whether the norsteroid conjugates produced and excreted in different conditions would show a pattern that might be linked to their origin. The $\delta^{13}C$ values of the metabolites produced after the ingestion of edible parts of non-castrated pig were measured by isotope ratio mass spectrometry. Our results indicated that it is not possible to determine the origin of the urinary metabolites based soley upon the evaluation of the different metabolites and conjugates. GC/C/IRMS is the only method capable of distinguishing between the exogenous and endogenous origin of the metabolites

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Anal Chim Acta 2009 **637** (1-2) 247

Detection of anabolic androgenic steroid abuse in doping control using mammalian reporter gene bioassays

Often detected as drugs in sport doping control are anabolic androgenic steroids (AAS) which are a class of steroid hormones related to the male hormone testosterone. Due to their similar structure or derivation from natural male hormones, AAS share the activation of the androgen receptor (AR) as a common mechanism of action. The mammalian androgen responsive reporter gene assay (AR) which measures compounds interacting with the AR may be used for the analysis of AAS without the necessity of prior knowledge of their chemical structure. Conversely, current chemical-analytical approaches may

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have trouble in detecting compounds with unknown structures, such as designer steroids. This paper demonstrates that AAS prohibited in sports and potential designer AAS may be detected with the AR CALUX bioassay. In addition, steroid activities of AAS may be found with additional mammalian bioassays for other types of steroid hormones. Mixtures of AAS were noted to act additively in the AR reporter gene assay indicating that it is possible to use this method for complex mixtures as are found in doping control samples, including mixtures that are a result of multi drug use. To determine whether mammalian reporter gene assays could be used for the detection of AAS in urine samples, background steroidal activities were measured. AAS-spiked urine samples, mimicking doping positive samples, demonstrated significantly greater androgenic activities than unspiked samples. GC-MS analysis of endogenous androgens and AR CALUX bioassay analysis of urine samples demonstrated how a combined chemical-analytical and bioassay approach may be employed to identify samples containing AAS. In terms of doping control purposes, the results indicate that the AR CALUX bioassay, in addition to chemical-analytical methods, is an important technique for the analysis of AAS

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Steroids 2009 **74** (3) 322

Identification of steroid isoxazole marketed as a designer supplement

Orastan-A from Gaspari Nutrition was analyzed for its steroid content. The contents label stipulates "5α-androstano[2,3-*c*]furazan-17β-tetrahydropyranol ether", also called furazadrol-THP ether. However, GC-MS analyses of the liberated steroids (after extraction from the capsule matrix and cleavage of the THP ether, TMS-derivative and underivatized) revealed mass spectra of two components, both inconsistent with the supposed contents. Consequently, they were analysed by different several techniques such as mass spectrometry, nuclear magnetic resonance spectroscopy and X-ray crystal structure analysis. The analytes were identified as 17β-hydroxyandrostano[3,2-*c*]isoxazole and -[2,3-*d*]isoxazole

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Biomed Chromatogr 2009 **23** (8) 873

Quantification of testosterone undecanoate in human hair by liquid chromatography-tandem mass spectrometry

Testosterone undecanoate (T-C11) can be used by athletes in order to improve performance. After oral intake, T-C11 is rapidly metabolized, hampering discrimination between exogenous and endogenous testosterone. A possible alternative is to detect the intact ester in hair. A method based on liquid chromatography-tandem mass spectrometry was developed for the determination of T-C11 in hair. The sample procedure consisted of digestion of 200 mg of pulverized hair with tris(2-carboxyethyl)phosphine hydrochloride and liquid-liquid extraction with *n*-pentane. Several parameters such as the mobile phase, the ionization source and the washing step were optimized. The method was validated at different spiked levels obtaining satisfactory values for accuracy (between 92 and 102%) with relative standard deviations lower than 7% and a limit of detection of 0.2 ng/g. The applicability of the method was checked by the analysis of three samples from patients using T-C11. A peak for the analyte was detected in all samples with concentrations between 0.4 and 8.4 ng/g

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Anal Chim Acta 2009 **637** (1-2) 305

Detection of anabolic steroids in dietary supplements: The added value of an androgen yeast bioassay in parallel with a liquid chromatography-tandem mass spectrometry screening method

A recombinant yeast cell that expresses the human androgen receptor (hAR) and yeast enhanced green fluorescent protein (yEGFP), the latter in response to androgens was previously constructed. Following exposure to testosterone, the concentration where half-maximal activation is reached (EC_{50}) was 50 nM. Eighteen different dietary supplements which had been tested for the presence of anabolic steroids by liquid chromatography-tandem mass spectrometry (LC-MS/MS) were subsequently screened for androgens. Positives in the bioassay were noted for 11 samples containing at least one anabolic steroid, with a concentration that was around or above 0.01/mgunit by LC-MS/MS. None of the 49 compounds screened for in LC-MS/MS were recorded from 7 samples. However, two of them were positive in the bioassay. Employing the bioassay as an off-line LC-detector and LC-time of flight-MS with accurate mass measurement was carried out in these two samples and revealed the presence of 4-androstene-3β,17β-diol and 5α-androstane-3β,17β-diol in the first and 1-testosterone in the second supplement. This result demonstrates the additional value of the bioassay when compared with a LC-MS/MS screening method alone

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J Liq Chromatogr Relat Technol 2009 **32** (8) 1107

Development of a validated HPLC method for the simultaneous determination of anabolic steroids in biological fluids

A method was developed and validated for the simultaneous determination of anabolic steroids: testosterone (TES), epitestosterone (EPI), and nandrolone (NAN) employing reversed phase high performance liquid chromatography (HPLC). The analytical column, Inertsil C8, 5 µm, 250 x 4mm, was operated at ambient temperature. Isocratic elution was performed using a mixture of 50% buffer solution CH3COOH 0.11% -CH3COONa 7.5 mmol/l, pH=4, 45% CH3CN and 5% CH3OH, at a flow rate of 1.1ml/min. UV detection was performed at 238 nm. The detection limits of the method were 2.4 ng for NAN, 3.6ng for TES, and 2.6ng for EPI in blood plasma, and 2.7ng for NAN, 1.1 ng for TES, and 3.8ng for EPI in urine, per 20 µl injection volume. Alprazolam was used as internal standard at a concentration of 2 ng/µl. Validation of the technique was performed both in terms of accuracy and precision. Intra-day assay (n=6) and inter-day assay (n = 3 x 6) and was found to be satisfactory, with high accuracy and precision results. Sample preparation employed solid phase extraction on Nexus cartridges with high recoveries. Analysis of urine samples of one female and 9 male volunteers were successfully analysed by the developed method

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Steroids 2009 **74** (3) 365

Substantial advantage of a combined Bayesian and genotyping approach in testosterone doping tests

A urinary testosterone/epitestosterone (T/E) ratio above 4.0 is considered suspicious of testosterone abuse. However, a deletion polymorphism in the gene coding for UGT2B17 is strongly associated with reduced testosterone glucuronide (TG) excretion in urine. Many individuals where the gene is absent would not reach a T/E ratio of 4.0 after testosterone intake. Therefore, it may be necessary to shift from population based- to individual-based T/E cut-off ratios using Bayesian inference. An individual's true negative baseline T/E ratio is required for longitudinal analysis. The object of this research was to determine whether it is possible to increase the sensitivity and specificity of the T/E test by addition of *UGT2B17* genotype information in a Bayesian framework. Fifty-five healthy male volunteers with either two, one or no allele (*ins/ins*, *ins/del* or *del/del*) of the *UGT2B17* gene were administered a single intramuscular dose of 500mg testosterone enanthate. Subsequently, urinary excretion of TG and the T/E ratio was measured during 15 days and a Bayesian analysis was conducted to calculate the individual T/E cut-off ratio. Following addition of genotype information, the program produced lower individual cut-off ratios in all *del/del* subjects and increased the sensitivity of the test considerably. Without knowledge of the *UGT2B17* genotype, it will be difficult, if not impossible, to distinguish between a true negative baseline T/E value and a false negative one. *UGT2B17* genotype information is vital, both to determine the initial cut-off ratio to use for an individual, and to increase the sensitivity of the Bayesian analysis

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J Chromatogr A 2009 **1216** (14) 2913

Analytical challenges in doping control: Comprehensive two-dimensional gas chromatography with time of flight mass spectrometry, a promising option

An investigation was conducted in respect of doping control screening based on the enhanced resolution of comprehensive two-dimensional (2D) gas chromatography hyphenated to time of flight mass spectrometry. Anabolic agents (clenbuterol, norandrosterone, epimetendiol, two methyltestosterone metabolites and 3'-hydroxystanozolol) contained in a spiked urine sample (2ng/ml) were identified. Particular emphasis was given to 3'-hydroxystanozolol, mainly considering the difficulty in its detection. By contrast with conventional GC-MS approaches that must employ single-ion monitoring, the GC x GC-TOFMS method enabled the identification through the deconvolution of the full mass spectrum and also resolved the co-eluted peaks of 3'-hydroxystanozolol and an endogenous component

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Steroids 2009 **74** (3) 359

Profiling of 19-norandrosterone sulfate and glucuronide in human urine: Implications in athlete's drug testing

The target metabolite in anti-doping testing to reveal the abuse of nandrolone or nandrolone prohormone is 19-norandrosterone (19-NA) as its glucuronide derivative. The sulfoconjugate form of 19-norandrosterone in human urine might be monitored additionally to provide further evidence of a doping with these steroids. Following administration of 19-nor-4-androstenedione (100mg) to 8 male subjects, sulfate and glucuronide derivatives of 19-norandrosterone together with 19-noretiocholanolone (19-NE) were assessed in spot urines. LC/MS/MS was performed for the direct quantification of sulfoconjugates, whereas a standard GC/MS method was employed for the assessment of glucuroconjugates in urine specimens. Whereas 19-NA glucuronide metabolite was always the most prominent, inter-individual variability of the excretion patterns was noted for both conjugate forms of 19-NA and 19-NE. However, the ratio of glucuro- to sulfoconjugate metabolites of 19-NA and 19-NE could not discriminate the endogenous *versus* the exogenous origin of the parent compound. Following ingestion of 100mg 19-nor-4-androstenedione, it was noted in the urine specimens that the sulfate conjugate of 19-NA was detectable over a longer period of time in respect of the other metabolites. These results suggest that more prominence should be paid to conjugates in order to deter a potential doping with norsteroids

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Steroids 2009 **74** (3) 315

Doping control analysis of trenbolone and related compounds using liquid chromatography-tandem mass spectrometry

A class of highly potent anabolic-androgenic steroids prohibited in sports according to the regulation of the World Anti-Doping Agency (WADA)includes trenbolone (17β-hydroxy-estra-4,9,11-trien-3-one) and its derivatives such as 17α-methyltrenbolone. The method of choice for the detection of these analytes in sports drug testing has been liquid chromatography-tandem mass spectrometry (LC-MS/MS) due to their marginal gas chromatographic properties but excellent proton affinities resulting from a large and conjugated pi-electron system. A sensitive and robust analytical method based on an enzymatic hydrolysis of target compounds, liquid-liquid extraction and subsequent LC-MS/MS measurement has become necessary following recent findings of trenbolone and methyltrenbolone in doping control urine samples of elite athletes. Diagnostic product ions produced by collision-induced dissociation of protonated molecules were found at *m/z* 227, 211, 199 and 198, which enabled targeted screening using multiple reaction monitoring. For 7 model compounds (trenbolone, epitrenbolone, methyltrenbolone, ethyltrenbolone, propyltrenbolone, 17-ketotrenbolone and altrenogest), the established method was validated for specificity, lower limits of detection (0.3-3ng/ml), recovery (72-105%), intraday and interday precision (< or =20%)

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J Anal Chem Engl Tr 2009 **64** (1) 31

Detection of oxandrolone and its metabolite in urine by high-performance liquid chromatography-high-resolution mass spectrometry with atmospheric pressure chemical ionization and Orbitrap detection after ceasing drug administration

Up to two weeks after ceasing its administration, oxandrolone may be detected by high-performance liquid chromatography-high-resolution mass spectrometry with atmospheric pressure chemical ionization and Orbitrap detection. The mass accuracy in the analysis of real samples was 2 ppm. The detection limits for oxandrolone and its metabolite were 3 and 1 pg/ml, respectively (signal-to-noise ratio of 10)

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Steroids 2009 **74** (3) 329

Doping in sport-1. Excretion of 19-norandrosterone by healthy women, including those using contraceptives containing norethisterone

The World Anti-Doping Agency (WADA) prohibits the use of the anabolic steroid nandrolone and its prohormones in sport. However, 19-norandrosterone (19-NA), the principal urinary metabolite, is often reported in samples. The urinary concentrations of 19-NA which may be found in women who are not using anabolic steroids, including those using oral contraceptives containing the 19-nor progestogen norethisterone has been little investigated. The reporting threshold for 19-NA for females was lowered from 5 to 2ng/ml in 2004 by WADA. This large-scale investigation was prompted by the lack of any substantial data on 19-NA excretion in women. Analysis was made of 19-NA in single untimed urine samples were collected from 1202 female volunteers, 38 of whom were taking norethisterone containing contraceptives. None of the subjects was a competitive athlete and pregnancy had been excluded by a urinary test for human chorionic gonadotropin (hCG). The 19-NA reporting threshold having a concentration of 4.1ng/ml was exceeded in only one sample and this was from a user of a norethisterone-containing contraceptive

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Steroids 2009 **74** (3) 335

Doping in sport-2. Quantification of the impurity 19-norandrostenedione in pharmaceutical preparations of norethisterone

The World Anti-Doping Agency (WADA) prohibits the use of 19-norandrostenedione, an anabolic steroid, in sport. 19-Norandrostenedione was discovered in norethisterone tablets in measurable amounts and this instigated research to develop an assay to quantify this steroid. Using [3,4-$^{13}C_2$]-19-norandrostenedione as the internal standard, an assay was developed employing isotope dilution and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify 19-norandrostenedione in norethisterone formulations. The results indicated amounts up to 1.01+/-0.01μg (mean+/-S.E.M.) per tablet in those containing 5mg of norethisterone or norethisterone acetate (0.02%, w/w) and up to 0.5+/-0.01μg (mean+/- S.E.M.) per tablet (0.05%, w/w) in oral contraceptive tablets containing 0.35-1.5mg of norethisterone or norethisterone acetate. However, the British Pharmacopoeia limit of 0.1% for this impurity was not exeeded in any tablet

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Steroids 2009 **74** (3) 341

Doping in sport-3. Metabolic conversion of oral norethisterone to urinary 19-norandrosterone

Prima facie evidence of administration of nandrolone or other 19-norsteroid in a competitor's urine sample is the detection of 19 norandrosterone (19-NA). However, norethisterone, a progestogen used for menstrual disorders and for hormonal contraception, also results in the excretion of 19-NA that may exceed the laboratory reporting threshold of 2ng/ml and thus confound results. An investigation was conducted of the contribution of norethisterone to urinary 19-NA with and without 19-norandrostenedione, a known norethisterone tablet impurity. Formulations containing, either <2ng or 1μg 19-norandrostenedione impurity per 5mg of norethisterone, administered to female volunteers (n=10) in doses comparable to those used for menstrual disorders (5mg three times daily for 10 days), produced maximal 19-NA concentrations of 51 and 63ng/ml, respectively. The greatest concentration of 19-NA, 2h post-administration of a single 1μg dose of 19-norandrostenedione, was 2.4ng/ml. These results demonstrate unambiguously that norethisterone is metabolized to 19-NA and that there is only a minor contribution from the impurity 19-norandrostenedione. Administration to women (n=30) of a single contraceptive tablet containing norethisterone (1mg) with one of the highest proportions of the impurity 19-norandrostenedione (approximately 0.5μg, 0.05%, w/w) produced a urinary 19-NA concentration of 9.1ng/ml, with a maximum concentration ratio of 19-NA to the norethisterone metabolite 3α,5β-tetrahydronorethisterone of 0.36. Data is provided that should mitigate the requiement for time-consuming follow-up investigations to consider whether doping with 19-norandrogens has occurred

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Steroids 2009 **74** (3) 369

Steroid isotopic standards for gas chromatography-combustion isotope ratio mass spectrometry (GCC-IRMS)

A recognized test to detect illicit doping with synthetic testosterone is carbon isotope ratio (CIR) analysis of urinary steroids using gas chromatography-combustion isotope ratio mass spectrometry (GCC-IRMS). However, there are currently no universally employed steroid isotopic standards (SIS). A protocol was employed to prepare isotopically uniform steroids for use as a calibrant in GCC-IRMS that might be analyzed under the same conditions as used for steroids extracted from urine. Two separate SIS containing a mixture of steroids were produced and coded CU/USADA 33-1 and CU/USADA 34-1, containing acetates and native steroids, respectively. CU/USADA 33-1 contains 5α-androstan-3β-ol acetate (5α-A-AC), 5α-androstan-3α-ol-17-one acetate (androsterone acetate, A-AC), 5β-androstan-3α-ol-11, 17-dione acetate (11-ketoetiocholanolone acetate, 11k-AC) and 5α-cholestane (Cne). CU/USADA 34-1 contains 5β-androstan-3α-ol-17-one (etiocholanolone, E), 5α-androstan-3α-ol-17-one (androsterone, A), and 5β-pregnane-3α, 20α-diol (5βP). Samples of each mixture were prepared and dispensed into a set of approximately 100 ampoules

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employing a protocol carefully designed to minimize isotopic fractionation and contamination. A natural gas reference material, NIST RM 8559, traceable to the international standard Vienna PeeDee Belemnite (VPDB) was utilised to calibrate the SIS. Absolute $\delta^{13}C$(VPDB) and $\delta\delta^{13}C$(VPDB) values from randomly selected ampoules from both SIS demonstrate uniformity of steroid isotopic composition within measurement reproducibility, SD($\delta^{13}C$) < 0.2%. This technique for production of isotopic steroid mixtures results in consistent standards with isotope ratios traceable to the relevant international reference material

4 Peptides

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Clin Chem 2009 **55** (3) 445

High-sensitivity chemiluminescence immunoassays for detection of growth hormone doping in sports

Whereas recombinant human growth hormone (rhGH) is abused to improve sports performance, adequate routine doping tests are lacking. Serum hGH isoform composition analysis has been shown to be effective in detecting rhGH doping. Selective immunoassays for isoform analysis with potential utility for screening and confirmation in doping tests have been developed and validated. Monoclonal antibodies with specificity for pituitary hGH (phGH) or rhGH were employed to establish 2 pairs of sandwich-type chemiluminescence assays with differential recognition of rhGH (recA and recB) and phGH (pitA and pitB). Specimens from volunteers before and after administration of rhGH were analysed. Ratios between the respective rec- and pit-assay results were produced. Functional sensitivities were <0.05 µg/l, with intra- and interassay imprecision < or =8.4% and < or =13.7%, respectively. Rec/pit ratios (median range) were 0.84 (0.09-1.32)/0.81 (0.27-1.21) (recA/pitA) and 0.68 (0.08-1.20)/0.80 (0.25-1.36) (recB/pitB), with no sex difference in 2 independent cohorts of healthy subjects. Ratios (median SD) increased after a single injection of rhGH, reaching 350% (73%) (recA/pitA) and 400% (93%) (recB/pitB) of baseline ratios in 20 recreational athletes. Following a moderate dose (0.033 mg/kg), mean recA/pitA and recB/pitB ratios remained significantly elevated for 18 h (men) and 26 h (women). However, after high-dose rhGH (0.083 mg/kg), mean rec/pit ratios remained increased for 32 h (recA/pitA) and 34 h (recB/pitB) in men and were still increased after 36 h in women. Detection of a single injection of rhGH was possible for up to 36 h when the sensitive chemiluminescence assays with preferential recognition of phGH or rhGH were employed

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Clin Ther 2009 **31** (2) 336

Impact of illegal trade on the quality of epoetin in Thailand

Counterfeit medicines pose a serious problem in developing countries according to reports from the World Health Organization. Drug smuggling in Thailand has been indicated following an investigation of anti-erythropoietin antibody-mediated pure red cell aplasia. Therefore, the authenticity and quality of epoetin α samples in Thailand was investigated to mitigate any potential serious safety implications. Epoetin α-prefilled syringes were tested from the pharmacies at 2 major hospitals (62 samples), 8 retail pharmacies (41 samples), and Thai authorities (30 samples confiscated from smugglers at 2 airports, and 6 samples from a condominium used by smugglers). Samples were analysed against the European Union Pharmacopeia specifications for aggregate content in epoetins of <2%. The veracity of epoetin α distribution logistics, coldchain storage (maintenance at 2°C-8°C), primary and secondary packaging components (eg, batch number, expiration date, appearance, letter size), and company's covert features (eg, nature of the ink, type and quality of the paper, other covered features) were also analysed. The primary data related to protein aggregate content, determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blotting; and isoform distribution, analysed by isoelectric focusing and Western blotting. Company cold-chain and authorized distribution channels for epoetin α met all quality standards and simialrly all epoetin α samples obtained from the hospital pharmacies. However, analyses determined that some samples were being smuggled or sold illegally through certain unauthorized retail pharmacies. Both airport and the condominium outlets stored epoetin α samples improperly at room temperature. Aggregate levels were greater than the specification of <2% in 11 samples from 2 of the retail pharmacies (range, 1.2%-3.1%), 15 samples from the Dongmuang Airport (range, 2.2%-17.0%), and all 6 samples from the condominium (range, 10.5%-19.8%). Epoetin α from the 2 hospitals, 8 retail pharmacies, and Suvarnabhumi Airport had the authentic 6 isoform bands. However, those from Dongmuang Airport and the condominium appeared to have the 6 characteristic bands, but positive confirmation was impaired due to band smearing caused by a high level of aggregates. All aspects of primary and secondary packaging were found to be genuine. Some evidence was discovered that some epoetin α was smuggled into Thailand without proper cold storage, contained high levels of protein aggregates, and were sold illegally through certain retail pharmacies. Such unauthorized products have been stopped from reaching patients following Thai authority intervention. Illegal cross-border smuggling of biopharmaceuticals without proper cold storage poses serious safety implications for patients in developing countries and efforts must be made to prevent it

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J Chromatogr A 2009 **1216** (12) 2574

Analysis of recombinant human erythropoietin and novel erythropoiesis stimulating protein digests by immunoaffinity capillary electrophoresis-mass spectrometry

A method to confirm the presence of recombinant human erythropoietin (rhEPO) in solution *via* detection of a specific peptide marker by immunoaffinity capillary electrophoresis-mass spectrometry (IA-CE-MS) is described. In addition to the carbohydrate content, the amino acid sequence of novel erythropoiesis stimulating protein (NESP) differs from human erythropoietin (hEPO) at five positions (Ala30Asn, His32Thr, Pro87Val, Trp88Asn, and Pro90Thr). Following digestion of both glycoproteins in solution by trypsin and PNGase F, two specific proteotypic peptides, EPO (77-97) and NESP (77-97) which differ in three amino acids, were selected as rhEPO and NESP markers, respectively. IA-CE-MS was employed to analyse both digests and their mixtures. The IA stationary phase was constructed from a custom-made polyclonal anti-EPO (81-95) antibody immobilized on a solid support of CNBr-Sepharose 4B and was packed in a microcartridge near the inlet of the separation capillary. The antibody was specific to a synthetic peptide EPO (81-95). Therefore, only the proteotypic peptide EPO (77-97) was retained. This was eluted, separated by electrophoresis and detected by MS. The technique was sufficiently specific to confirm the presence of rhEPO in solution. Whereas the limits of detection for the peptide marker were similar to those obtained with CE-MS (a few mg/l), the results indicate the potential of this novel approach to detect in the future rhEPO and its analogues selectively and unambiguously at the levels expected in biological fluids

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Int J Sports Med 2009 **30** (2) 80

Identification of darbepoetin in human plasma by liquid chromatography coupled to mass spectrometry for doping control

An analog of recombinant human erythropoietin (rhEPO), darbepoetin α (DPO) or novel erythropoiesis stimulating protein (NESP) is abused as a blood doping agent to improve in human sports performance. Indisputable identification of DPO in human plasma following extraction of the analyte from plasma by immunoaffinity separation with anti-rhEPO antibodies, digested by trypsin followed by PNGase F, and determined by liquid chromatography coupled to tandem mass spectrometry. The deglycosylated tryptic peptide, T_9, was employed in DPO identification using liquid chromatographic retention time and major product ions of the T_9 peptide. The limit of detection of this method for DPO was 0.1 ng/ml in plasma, and that of identification was 0.2 ng/ml. This technique provides "mass fingerprints" for identification of DPO in human plasma samples. No false positives were noted. This technique cannot be employed to identify rhEPO in human plasma because it cannot differentiate rhEPO from endogenous EPO. However, it is the first successful attempt towards establishing a reliable and selective method for definitive identification of exogenously administered EPOs in doping control analyses

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Rapid Commun Mass Spectrom 2009 **23** (19) 3173

High-throughput ultra-high-performance liquid chromatography/tandem mass spectrometry quantitation of insulin-like growth factor-I and leucine-rich -2-glycoprotein in serum as biomarkers of recombinant human growth hormone administration

Insulin-like growth factor-I (IGF-I) is a known biomarker of recombinant human growth hormone (rhGH) abuse, and is also used clinically to confirm acromegaly. The protein leucine-rich α-2-glycoprotein (LRG) was recently identified as a putative biomarker of rhGH administration. The combination of

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an ACN depletion method and a 5-min ultra-high-performance liquid chromatography/tandem mass spectrometry (uHPLC/MS/MS)-based selected reaction monitoring (SRM) assay detected both IGF-I and LRG at endogenous concentrations. Four eight-point standard addition curves of IGF-I (16-2000 ng/ml) demonstrated good linearity (r^2 = 0.9991 and coefficients of variance (CVs) <13%). Serum samples from two rhGH administrations were extracted and their uHPLC/MS/MS-derived IGF-I concentrations correlated well against immunochemistry-derived values. Combining IGF-I and LRG data improved the separation of treated and placebo states compared with IGF-I alone, further strengthening the hypothesis that LRG is a biomarker of rhGH administration. Artificial neural networks (ANNs) analysis of the LRG and IGF-I data demonstrated an improved model over that developed using IGF-I alone, with a predictive accuracy of 97%, specificity of 96% and sensitivity of 100%. Receiver operator characteristic (ROC) analysis gave an AUC value of 0.98. This study demonstrates the first large scale and high throughput uHPLC/MS/MS-based quantitation of a medium abundance protein (IGF-I) in human serum. Furthermore, the data we have presented for the quantitative analysis of IGF-I suggest that, in this case, monitoring a single SRM transition to a trypsin peptide surrogate is a valid approach to protein quantitation by LC/MS/MS

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J Proteome Res 2009 **8** (2) 1071
Identification of human pituitary growth hormone variants by mass spectrometry
Doping control analysis of plasma samples employs the heterogeneity of human endogenous growth hormone (GH) to distinguish it from the homogeneous recombinant analogue. Pituitary GH variants were analysed by gel electrophoresis and mass spectrometry. In addition to 22 and 20 kDa isoforms, fragments of 9 and 12 kDa were identified and a glycosylated 23 kDa GH variant was demonstrated to bear a HexHexNac 2 NeuAc modification presumably located at Thr 60

5 Diuretics

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J Mass Spectrom 2009 **44** (10) 1538
Formation of the unusual $[M+H-11\ Da]^+$ ion peak in the collision-induced dissociation mass spectrum of $[M+H]^+$ ion of hydrochlorothiazide
No abstract available

6 CNS Agents

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Eur J Mass Spectrom 2009 **15** (4) 507
Doping control analysis of metamfepramone and two major metabolites using liquid chromatography-tandem mass spectrometry
Metamfepramone (2-dimethylamino-1-phenylpropan-1-one, dimethylpropion) is a sympathomimetic agent widely employed in the treatment of the common cold or hypotonic conditions. It has stimulating properties and its rapid metabolism producing major degradation products such as methylpseudoephedrine and methcathinone, it has been considered relevant for doping controls by the World Anti-Doping Agency (WADA). The rapid metabolism of metamfepramone confounds its analysis but the metabolites methylpseudoephedrine and methcathinone can be detected. The discovery of the metabolites in particular facilitates the inference of metamfepramone administration. In order to expedite sports drug testing procedures, metamfepramone, methylpseudoephedrine and methcathinone were characterized using electrospray ionization-high resolution/high accuracy mass spectrometry. A technique employing liquid chromatography/tandem mass spectrometry was developed that allowed the analysis of the three compounds by direct injection of 2 µl urine specimens. The assay was validated with regard to specificity, lower limits of detection (2-10 ng/ml, intraday and interday precision (3-17%) and ion suppression/enhancement effects. The technique was employed to verify or falsify suspicious signals observed in routine screening procedures based on gas chromatography/mass spectrometry and produced a positive finding concerning a metamfepramone administration in an authentic doping control sample. Whereas metamfepramone was not detected, the evidential metabolites methylpseudoephedrine and methcathinone were considered sufficient to infer the application of the prohibited drug

7 Equine

You YW, Uboh CE*, Soma LR, Guan F, Li XQ, Rudy JA, Chen JW// *West Chester Univ, Dept Chem, PA Equine Toxicology & Res Ctr, West Chester, Pa 19382, USA
J Anal Toxicol 2009 **33** (1) 41
Screening, quantification, and confirmation of phenylbutazone and oxyphenbutazone in equine plasma by liquid chromatography-tandem mass spectrometry
Screening, quantification, and confirmation of phenylbutazone and oxyphenbutazone in equine plasma has been achieved with a sensitive liquid chromatographic-tandem mass spectrometric method. Analytes were isolated from plasma by liquid-liquid extraction followed by separation in a reversed-phase column and identification by mass spectrometry with selected reaction monitoring in negative electrospray ionization mode. Extraction recovery for both analytes was >80%. Limits of detection, quantification, and confirmation for both analytes were 0.01 µg/ml (S/N>or= 3), 0.05 µg/ml, and 0.05 µg/ml, respectively. The assay with d9-labeled phenylbutazone as internal standard (IS) was linear over a range of 0.05-20 µg/ml (r^2>0.995). In terms of coefficient of variation, intra- and interday precision was less than 15%. Intra- and interday accuracy (bias%) was within 80-120%. Decreased analyte signal intensity was noted following hemolysis of red blood cells but did not affect quantification results because an isotope-labeled IS was used. Analytes were stable in plasma for 24 h at room temperature, 9 days at 4ºC, and 45 days at -20ºC and -70ºC. Screening, quantification, and confirmation of phenylbutazone in post-competition plasma samples obtained from racehorses was achieved successfully. The technique is simple, rapid, and reliably reproducible

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Chromatographia 2009 **69** (1-2) 59
Enantiomeric composition tests of ketoprofen in equine plasma and urine as diastereomeric (*S*)-(-)-1-phenylethylamides by achiral GC-MS
By ultilising the determination of diastereomeric (*S*)-(-)-1-phenylethylamidation with achiral gas chromatography-mass spectrometry in selected ion monitoring mode, the enantiomeric composition of ketoprofen in equine plasma and urine after administration of a commercial racemic ketoprofen product was determined. The technique showed linearity (r > 0.9986) over the range tested (5.0-5,000 ng), with acceptable precision (% RSD < 9.5) and accuracy (% RE = -3.7-7.3). The perecentage of (*S*)-ketoprofen in plasma after 6.0 h and in urine after 71.0 h increased progressively to final values of 67.3 ± 0.1 and 91.9 ± 2.2%, respectively, attributable to the inversion of (*R*)-ketoprofen to (*S*)-ketoprofen

8 Recreational Drugs - General

Berg T, Lundanes E, Christophersen AS, Strand DH// Norwegian Inst Publ Hlth, Div Forensic Toxicology & Drug Abuse, Oslo, Norway
J Chromatogr B 2009 **877** (4) 421
Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC-MS/MS
The determination of opiates (morphine, codeine, 6-monoacetylmorphine (6-MAM), pholcodine, oxycodone, ethylmorphine), cocaine and benzoylecgonine in urine by a fast and selective ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) technique has been developed and validated. Sample preparation was achieved using solid phase extraction (SPE) on a mixed mode cation exchange (MCX) cartridge. A basic mobile phase consisting of 5mM ammonium bicarbonate, pH 10.2, and methanol (MeOH) was identified for optimized chromatographic performance with repeatable retention times, narrow and symmetrical peaks, and focusing of all analytes at the column inlet at gradient start. Positive electrospray ionization (ESI^+) MS/MS detection was performed with a minimum of two multiple reaction monitoring (MRM) transitions for each analyte. Deuterium labelled-internal standards were employed for six of the analytes. Between-assay retention time repeatabilities (n=10 series, 225 injections in total) had relative standard deviation (RSD) values within 0.1-0.6%. Limit of detection (LOD) and limit of quantification (LOQ) values were in the range 0.003-0.05 µM (0.001-0.02 µg/ml) and 0.01-0.16 µM (0.003-0.06 µg/ml), respectively. The RSD values of the between-assay repeatabilities of concentrations were <or=10% at five concentration levels for all analytes, except for pholcodine. Determination of the retention times of 96 drugs and internal standards in total was employed to determine specificity. Co-eluting compounds were in all cases separated by the MS/MS detection. No or only minor matrix effects were noted. Total run time,

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including injection and equilibration time was 5.7 min. The technique has been routinely employed at the Norwegian Institute of Public Health (NIPH) since August 2007 for qualitative detection of opiates, cocaine and benzoylecgonine in more than 2000 urine samples with two replicates of each sample

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J Chromatogr A 2009 **1216** (15) 3078

Simultaneous ultra-high pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater

The simultaneous quantification and confirmation of 11 basic/acidic illicit drugs and relevant metabolites in surface and urban wastewater at ng/l levels has been determined following development of an ultra-high-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) technique. Sample pre-treatment consists of a solid-phase extraction using Oasis MCX cartridges. Deuterated compounds of analytes were employed as surrogate internal standards (except for norbenzoylecgonine and norcocaine) to compensate for possible errors resulting from matrix effects and those associated with the sample preparation procedure. Following SPE enrichment, selected drugs were separated within 6min under UHPLC optimized conditions. To efficiently combine UHPLC with MS/MS, a fast-acquisition triple quadrupole mass analyzer (TQD from Waters) in positive-ion mode (ESI^+) was employed. The excellent selectivity and sensitivity of the TQD analyzer in selected reaction monitoring mode facilitated quantification and reliable identification at the LOQ levels. Acceptable recoveries (70-120%) and precision (RSD<20%) were produced for most compounds in different types of water samples, spiked at two concentration levels [limit of quantification (LOQ) and 10LOQ]. Thus, surface water was spiked at 30 ng/l and 300 ng/l (amphetamine and amphetamine-like stimulants), 10 ng/l and 100 ng/l (cocaine and its metabolites), 300 ng/l and 3000 ng/l (tetrahydrocannabinol-COOH). Recovery experiments in effluent and influent wastewater were conducted with spiking levels of three and fifteen times higher than the levels spiked in surface water, respectively. The validated method was employed with urban wastewater samples (influent and effluent). The production of three selected reaction monitoring transitions per analyte facilitated positive findings to be confirmed by accomplishment of ion ratios between the quantification transition and two additional specific confirmation transitions. Drug consumption generally increased at the weekends and whilst an important musical event was occurring. The highest concentration levels were 27.5 µg/l and 10.5 µg/l, and these corresponded to 3,4-methylenedioxymethamphetamine (MDMA, or Ecstasy) and to benzoylecgonine (a cocaine metabolite), respectively. Wastewater treatment plants showed good removal efficiency (>99%) for low levels of illicit drugs in water. However, some difficulties were noted when high drug levels were present in wastewaters

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Anal Bioanal Chem 2009 **393** (8) 1977

A liquid chromatography tandem mass spectrometry method for the simultaneous quantification of 20 drugs of abuse and metabolites in human meconium

Twenty cocaine, amphetamine, opiate, and nicotine analytes in meconium, the first neonatal feces were simultaneously quantified by liquid chromatography tandem mass spectrometry and the technique was validated. Methanol homogenization and solid phase extraction were included in specimen preparation. Two injections were necessary to achieve sufficient sensitivity and linear dynamic range. Linearity ranged from 0.5-25 up to 500 ng/g (250 ng/g *p*-hydroxymethamphetamine), and correlation coefficients were >0.996. Imprecision was <10.0% CV, analytical recovery 85.5-123.1%, and extraction efficiencies >46.7% at three concentrations across the linear range. In spite of significant matrix effects of -305.7-40.7%, effects were similar for native and deuterated analytes. No carryover, endogenous or exogenous interferences were noted, with analyte stability at room temperature, 4ºC, and -20ºC and on the autosampler >70%, except for 6-acetylmorphine, hydrocodone, oxycodone, and morphine. Analysis of meconium from drug-exposed neonates was employed to demonstrate method applicability

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Anal Bioanal Chem 2009 **393** (8) 1957

Simultaneous quantification of nicotine, opioids, cocaine, and metabolites in human and fetal postmortem brain by liquid chromatography tandem mass spectrometry

The simultaneous quantification of nicotine, cocaine, 6-acetylmorphine (6AM), codeine, and metabolites in 100 mg fetal human brain was determined by LCMSMS and the technique validated. Analytes were resolved on a Hydro-RP analytical column with gradient elution following homogenization and solid-phase extraction. Empirically determined linearity was from 5-5,000 pg/mg for cocaine and benzoylecgonine (BE), 25-5,000 pg/mg for cotinine, ecgonine methyl ester (EME) and 6AM, 50-5000 pg/mg for *trans*-3-hydroxycotinine (OH-cotinine) and codeine, and 250-5,000 pg/mg for nicotine. Potential endogenous and exogenous interferences were resolved. Intra- and inter-assay analytical recoveries were > or = 92%, intra- and inter-day and total assay imprecision were < or = 14% RSD and extraction efficiencies were > or = 67.2% with < or = 83% matrix effect. A postmortem fetal brain containing 40 pg/mg cotinine, 65 pg/mg OH-cotinine, 13 pg/mg cocaine, 34 pg/mg EME, and 525 pg/mg BE was employed to demonstrate method applicability. This validated technique is useful for determination of nicotine, opioid, and cocaine biomarkers in brain

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Clin Toxicol 2009 **47** (3) 253

Increasing burden of pill identification requests to US poison centers

Pill identification (PID) calls to poison centers have been increasing. A retrospective review of information calls reported to U.S. poison centers for 2002-2007 has been conducted. The total number of information calls increased by 44%, whereas calls related to human exposures increased by 4.3%. The subcategory "pill identification" was exclusively responsible for the increase in information calls with an increase of greater than 90%. During the 6 years, PID requests from the public, police, and healthcare facility changed by +110, +144, and -24%, respectively. PID requests from the public, police, and healthcare facility were 78, 12 and 10%, respectively. However, other information calls showed a decrease or no change with calls for poison information (-17%), medical information (-2.5%), and drug information (non-PID) (+1%). Of all calls to U.S. poison centers, 25% are now to identify a pill unrelated to an exposure. Of all identified pills, 64% were drugs with abuse potential. Whereas drugs with abuse potential are less than 4% of pharmaceutical sales, more than 60% of all PID requests involved drugs with abuse potential. The results indicate that PID is strongly driven by interest in drugs with abuse potential. PID calls are increasing dramatically and straining limited poison center resources. Most requests for identification of an unknown pill involved drugs with abuse potential

9 Stimulants

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Forensic Sci Int 2009 **185** (1-3) 67

Comparison of GC-MS and GC-IRD methods for the differentiation of methamphetamine and regioisomeric substances

The differentiation of regioisomeric phenethylamines related to methamphetamine was performed by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-infrared detection (GC-IRD). Essentially equivalent mass spectra were produced by a total of five regioisomeric phenethylamines (methamphetamine and four regioisomers). This unique set of five phenethylamines with the same molecular weight and elemental composition produce major mass spectral fragments at equivalent mass. Characteristic individual fragment ions allowing structural differentiation among these regioisomers was provided by trifluoroacetyl derivatives of the primary and secondary amines. Vapor phase infrared spectra produced by capillary gas chromatography distinguished the compounds without the necessity of derivatization. Regioisomeric phenethylamines may be well determined by GC and the elution order is generally determined by the degree of molecular linearity

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J Brazil Chem Soc 2009 **20** (2) 348

Simultaneous quantification of amphetamines and ephedrines in urine by GC/MS using analytical-grade acetic anhydride/pyridine as derivatizing reagents: A suitable approach to reduce costs of routine analyses

The simultaneous analyses of different amphetamines and ephedrines in urine employing analytical-grade acetic anhydride/pyridine as derivatizing reagents by GC/MS was developed and validated. Solid-phase extraction was performed on the samples. These were subsequenlty derivatizated and analyzed by GC/MS. The method showed a broad linear dynamic range (25-1000 ng/ml with $r^2 > 0.99$), high sensitivity (LODs of 0.0140 to 15.33 ng/ml and LOQs of 0.0466 to 51.10 ng/ml), good precision (CV < 6% for intra- and inter-assays), and excellent extraction recovery (87 to 96%) for all the compounds studied. Real samples of human urine which were previously determined to contain at least one of such drugs wereanalysed following validation of the technque. In

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all the samples, the amphetamines and ephedrines were immediately quantified, demonstrating that the combination of acetic anhydride and pyridine may be conveniently employed as a derivatizing agent

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Talanta 2009 **78** (2) 542
Effect of UV irradiation on detection of cocaine hydrochloride and crack vapors by IMIS and API-MS methods
The presence of illegal drug traces at the surface of mail items, documents, hands and banknotes is sometimes only possible *via* the detection of drug vapors and volatile products of their decomposition. The effect of UV irradiation on the sensitivity of a vapor phase detection of cocaine of different origin by a technology of ion mobility increment spectrometry (IMIS) is described. Increases the amplitude of IMIS signals by about eight fols was noted following UV irradiation on the surface of cocaine hydrochloride and crack. Ions resulting from the photolysis of tested cocaine samples using mass-spectrometry with atmospheric pressure ionization (API-MS) were analysed. An assumption is made about structural formula of volatile products of photolysis of crack and cocaine hydrochloride. From the results of API-MS and IMIS studies on photolysis of cocaine samples it is assumed that compound $C_{10}H_{15}NO_3$ with a molecular weight of 197 amu and ecgonidine methyl ester with a molecular weight of 181 amu are responsible for the increase of an amplitude of IMIS signals upon UV irradiation of samples of crack and cocaine hydrochloride

Lee S, Park Y, Yang W, Han E, Choe S, Lim M, Chung H// Natl Inst Sci Investigation, 331-1 Sinwol 7 dong, Yangcheon gu, Seoul 158 707, South Korea
Forensic Sci Int 2009 **185** (1-3) 59
Estimation of the measurement uncertainty of methamphetamine and amphetamine in hair analysis
Measurement uncertainties (MUs) were evaluated for the determination of methamphetamine (MA) and its main metabolite, amphetamine (AP) at the low concentrations (around the cut-off value of MA) in human hair in respect of the guidelines of the EURACHEM/CITAC Guide and "Guide to the expression of uncertainty in measurement (GUM)". MA and AP in hair were extracted by agitation with 1% HCl in methanol and then derivatization and quantification using GC-MS. The amount of MA or AP in the test sample, the weight of the test sample and the method precision, based on the equation to calculate the mesurand from intermediate values were major parameters contributing to their uncertainties. The concentrations of MA and AP in the hair sample with their expanded uncertainties were 0.66+/-0.05 and 1.01+/-0.06 ng/mg, respectively. These were acceptable to facilitate the successful application of the analytical technique. The largest contribution to the overall combined uncertainties of MA and AP, for each were the method precision and the weight of the hair sample

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Anal Chem 2009 **81** (8) 3051
Single quantum-dot-based aptameric nanosensor for cocaine
Recent developments in single-molecule detection, nanotechnology, and aptameric sensors show great promise for many potential applications. By functionalizing the surface of a quantum dot (QD) with aptamers which are capable of recognizing cocaine, and taking advantage of single-molecule detection and fluorescence resonance energy transfer (FRET) between 605QD and Cy5 and Iowa Black RQ, a single-QD-based aptameric sensor has been constructed that is capable of sensing the presence of cocaine through both signal-off and signal-on modes. When compared with the established aptameric sensors, this single-QD-based aptameric sensor is significantly better in respect of simple sample preparation, high sensitivity, and extremely low sample consumption. Following advances in the production of types of aptamers for small molecules, nucleic acids, metal ions, and proteins, this single-QD-based aptameric sensor could find wide application in forensic analysis, environmental monitoring, and clinic diagnostics

10 Hallucinogens

Awad T, DeRuiter J, Belal T, Clark CR*// *Auburn Univ, Sch Pharm, Dept Pharmacal Sci, Auburn, Al 36849, USA
J Chromatogr Sci 2009 **47** (4) 279
GC and mass spectral studies on acylated side chain regioisomers of 3-methoxy-4-methyl-phenethylamine and 4-methoxy-3-methyl-phenethylamine
The controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA) has a mass spectrum essentially equivalent to the side chain regioisomers of the 3-methoxy-4-methylphenethylamines and 4-methoxy-3-methylphenethylamines. All have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. In addition, the compounds in this study have ring substitutions in the same relative positions as 3,4-MDMA. The nonequivalence of the substituents (methoxy and methyl) yields two sets of compounds, 3-methoxy-4-methyl- and 4-methoxy-3-methyl-phenethylamines. The perfluoroacyl derivatives (pentafluoropropionylamides and heptafluorobutrylamides) of the primary and secondary regioisomeric amines were prepared and analysed by gas chromatography-mass spectrometry. The mass spectra for these derivatives are significantly individual and the unique fragment ions facilitate specific side chain identification. The heptafluorobutrylamide derivatives provide more fragment ions than the pentafluoropropionylamides for molecular individualization among these regioisomeric substances. A dimethyl polysiloxane stationary phase such as Rtx-1 produces excellent resolution of these acylated derivatives

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Clin Chem 2009 **55** (3) 454
Disposition of MDMA and metabolites in human sweat following controlled MDMA administration
Essential to the interpretation of sweat tests in drug treatment, criminal justice, and workplace programs, an nderstanding the excretion of 3,4-methylenedioxymethamphetamine (MDMA) and metabolites in sweat is required. Healthy volunteers (n = 15) with histories of MDMA use were given placebo, low (1.0 mg/kg), and high (1.6 mg/kg) doses of oral MDMA double-blind in random order. Following each dose, participants remained in the closed clinical research unit for up to 7 days. Before, during, and after controlled dosing, volunteers wore PharmChek sweat patches (n = 640). Patches were subjected to solid phase extraction and GC-MS for MDMA, methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA), and 4-hydroxy-3-methoxymethamphetamine (HMMA). Limits of quantification (LOQ) were 2.5 ng/patch for MDMA and 5 ng/patch for HMA, HMMA, and MDA. MDMA was the prominent analyte detected in 382 patches (59.7%), with concentrations up to 3007 ng/patch. MDA was detected in 188 patches (29.4%) at <172 ng/patch, whereas no HMMA or HMA was detected. A total of 224 patches (35.0%) and 60 patches (9.4%) were positive for MDMA and MDA, respectively, at the 25-ng/patch threshold proposed by the Substance Abuse and Mental Health Services Administration. In this controlled MDMA administration study, sweat testing was demonstrated to be an effective and reliable method for monitoring MDMA use. Some variability in sweat excretion indicates that results should be interpreted qualitatively rather than quantitatively. These data provide a basis for interpretation of MDMA sweat test results

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Traffic Inj Prev 2009 **10** (1) 16
The Victorian legislative framework for the random testing of drivers at the roadside for the presence of illicit drugs: An evaluation of the characteristics of drivers detected from 2004 to 2006
A new legislative framework for the random drug screening of drivers modeled on the successful random alcohol screening programme was introduced in December 2004 in Victoria, Australia. The new legislation prohibits driving while methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), and cannabis, Δ^9-tetrahydrocannabinol (THC) are at all present. The legislative authority to randomly drug test drivers for the presence of MA, MDMA, and THC by oral fluid (saliva) sample screening at the roadside was granted to the police. The new random drug testing legislative framework and the drug testing procedures currently in place in Victoria are described. In addition, data collected during the operation of the framework for the first two years since implementation in Victoria (December 2004-December 2006) is presented

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J Mass Spectrom 2009 **44** (10) 1423
Studies on the metabolism of the ^{9}-tetrahydrocannabinol precursor ^{9}-tetrahydrocannabinolic acid A (^{9}-THCA-A) in rat using LC-MS/MS, LC-QTOF MS and GC-MS techniques
In *Cannabis sativa*, Δ^9-tetrahydrocannabinolic acid-A (Δ^9-THCA-A) is the non-psychoactive precursor of Δ^9-tetrahydrocannabinol (Δ^9-THC). In fresh plant material, about 90% of the total Δ^9-THC is available as Δ^9-THCA-A. When heated (smoked or baked), Δ^9-THCA-A is only partially converted to Δ^9-THC and therefore, Δ^9-THCA-A can be detected in serum and urine of cannabis consumers. The aim of the presented study was to identify the

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metabolites of Δ^9-THCA-A and to examine particularly whether oral intake of Δ^9-THCA-A leads to *in vivo* formation of Δ^9-THC in a rat model. After oral application of pure Δ^9-THCA-A to rats (15 mg/kg body mass), urine samples were collected and metabolites were isolated and identified by liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and high resolution LC-MS using time of flight-mass spectrometry (TOF-MS) for accurate mass measurement. For detection of Δ^9-THC and its metabolites, urine extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The identified metabolites show that Δ^9-THCA-A undergoes a hydroxylation in position 11 to 11-hydroxy-Δ^9-tetrahydrocannabinolic acid-A (11-OH-Δ^9-THCA-A), which is further oxidized *via* the intermediate aldehyde 11-oxo-Δ^9-THCA-A to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinolic acid-A (Δ^9-THCA-A-COOH). Glucuronides of the parent compound and both main metabolites were identified in the rat urine as well. Furthermore, Δ^9-THCA-A undergoes hydroxylation in position 8 to 8-α- and 8-β-hydroxy-Δ^9-tetrahydrocannabinolic acid-A, respectively, (8α-hydroxy-Δ^9-THCA-A and 8β-hydroxy-Δ^9-THCA-A, respectively) followed by dehydration. Both monohydroxylated metabolites were further oxidized to their bishydroxylated forms. Several glucuronidation conjugates of these metabolites were identified. *In vivo* conversion of Δ^9-THCA-A to Δ^9-THC was not observed

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Talanta 2009 **77** (5) 1701
Rapid determination of ^{9}-tetrahydrocannabinol in saliva by polymer monolith microextraction combined with gas chromatography-mass spectrometry
Polymer monolith microextraction (PMME) combined with gas chromatography-mass spectrometry was employed to analyse Δ^9-tetrahydrocannabinol (THC) in saliva. Poly(methacrylic acid-*co*-ethylene glycol dimethacrylate) (p(MAA-*co*-EGDMA)) monolithic capillary column was selected as the extraction medium of PMME and exhibited high extraction capacity towards THC in saliva. For achievement of optimum PMME extraction performance, several PMME parameters were analysed including matrix pH, flow rate for extraction, sampling volume and elution solvent. Good extraction efficiency resulted under the optimal conditions with no matrix interference in the process of extraction and the subsequent GC-MS analysis. With the selected-ion monitoring (SIM) mode, the limit of detection (LOD) for THC was 0.68 ng/ml. The linearity range of the method was 3-300 ng/ml. Reproducibility of the method was excellent as demonstrated by intra- and inter-day precisions, yielding the relative standard deviations (R.S.D.s) less than 12%; recoveries higher than 89%. The technique was shown to be rapid, sensitive, and competently applied to the determination of THC in saliva samples

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Anal Bioanal Chem 2009 **393** (6-7) 1607
Hydrolysis of 3,4-methylenedioxymethamphetamine (MDMA) metabolite conjugates in human, squirrel monkey, and rat plasma
Insight into mechanisms of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") neurotoxicity may be gained by characterizing the formation of metabolites in different species (rat, squirrel monkey, and human). Two prominent MDMA metabolites, 3,4-dihydroxymethamphetamine (HHMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA), are conjugated with glucuronic or sulfuric acid. However, reference standards are not available and so quantification is only possible after cleavage of conjugates. Different concentrations of HHMA and HMMA were determined in human, squirrel monkey, and rat plasma specimens when acid or enzymatic cleavage was performed. The results indicate that these differences result from species-specific influences on conjugate cleavage. Acidic hydrolysis is necessary for successful analysis of free HHMA and HMMA in human or squirrel monkey plasma, while enzymatic hydrolysis with glucuronidase or sulfatase optimizes recovery of free HHMA and HMMA in rat plasma. Sulfate conjugates were more readily cleaved by acid hydrolysis and glucuronides by glucuronidase under optimal conditions

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J Chromatogr A 2009 **1216** (16) 3512
Using sweeping micellar electrokinetic chromatography to analyze ^{9}-tetrahydrocannabinol and its major metabolites
Sweeping micellar electrokinetic chromatography (sweeping-MEKC) has been employed in the simultaneous analysis of Δ^9-tetrahydrocannabinol (THC) and its major metabolites, 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH). The effects of several of the sweeping-MEKC parameters were monitored, including the proportion of organic modifier, the concentration of sodium dodecyl sulfate (SDS), the pH, and the sample injection volume, to optimize the separation process. The three analytes were analysed using an optimal buffer consisting of 25 mM citric acid/disodium hydrogenphosphate (pH 2.6) containing 40% methanol and 75 mM SDS. When separation parameters were optimised, the enrichment factors for THC, THC-COOH, and THC-OH when using sweeping-MEKC (relative to MEKC) were 77, 139, and 200, respectively. In standard solutions, the limits of detection (LODs) for the three compounds ranged from 3.87 to 15.2 ng/ml. Sweeping-MEKC method with solid-phase extraction to successfully analyse THC, THC-COOH, and THC-OH in human urine with acceptable repeatability. The LODs of these analytes in urine samples ranged from 17.2 to 23.3 ng/ml. Consequently, this sweeping-MEKC method is useful for analysing, with high sensitivity, the amounts of THC and its metabolites in the urine of suspected THC users

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J Anal Toxicol 2009 **33** (1) 51
Stabilization of urinary THC solutions with a simple non-ionic surfactant
A non-ionic surfactant, Tergitol, was investigated to reduce the requirement for special handling and storage of urinary solutions against adsorptive loss of metabolites of Δ^9-tetrahydrocannabinol (THC). The analytical process was notadversely affected by addition of surfactant up to 20 times the critical micelle concentration (CMC). However, at onlytwice CMC, the surfactant was found to mitigate adsorptive loss of THC analytes under a variety of storage and handling conditions including exposure to glass and plastic surfaces, after storage in a refrigerator or freezer, and at reduced pH, where adsorptive losses were expected to be significant. Micellar solubilization of analyte increased the assayed concentration on average by 10% with a range of 3 to 20%, depending on condition, relative to solutions without surfactant. Solutions with surfactant did not deviate in concentration by +/-20% over a 49-week period but those without surfactant did so by 21 weeks. Results indicate that addition of small amounts of non-ionic surfactant to solutions of urinary THC metabolites mitigate adsorptive losses during storage and handling. This is a simple method to improve both the accuracy and precision of analyte concentrations determined by gas chromatography-mass spectrometry

11 Narcotics

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Anal Bioanal Chem 2009 **394** (2) 513
Simultaneous quantification of buprenorphine, norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide in human placenta by liquid chromatography spectrometry
A LCMS technique has been developed and validated for the analysis of buprenorphine (BUP), norbuprenorphine (NBUP), buprenorphine glucuronide (BUP-Gluc), and norbuprenorphine glucuronide (NBUP-Gluc) in placenta. Quantification was accomplished by selected ion monitoring of *m/z* 468.4 (BUP), 414.3 (NBUP), 644.4 (BUP-Gluc), and 590 (NBUP-Gluc). BUP and NBUP were identified monitoring MS(2) fragments *m/z* 396, 414 and 426 for BUP, and 340, 364 and 382 for NBUP, and glucuronide conjugates monitoring MS^3 fragments *m/z* 396 and 414 for BUP-Gluc, and 340 and 382 for NBUP-Gluc. Linearity was 1-50 ng/g. Intra-day, inter-day and total assay imprecision (% RSD) were <13.4%, and analytical recoveries were 96.2-113.1%. Extraction efficiencies ranged from 40.7-68%, process efficiencies 38.8-70.5%, and matrix effect 1.3-15.4%. Limits of detection were 0.8 ng/g for all compounds. The placenta of an opioid-dependent pregnant woman receiving BUP treatment was analyzed. BUP was not detected but metabolite concentrations were NBUP-Gluc 46.6, NBUP 15.7 and BUP-Gluc 3.2 ng/g

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J Chromatogr B 2009 **877** (8-9) 833
Capillary zone electrophoresis with diode-array detection for analysis of local anaesthetics and opium alkaloids in urine samples
Two local anaesthetics (lidocaine and bupivacaine) and two opium alkaloids (noscapine and papaverine) were determined simultaneously by a capillary zone electrophoresis (CZE) with solid-phase extraction (SPE) procedure using Oasis HLB cartridges. Recoveries ranged from 81 to 107% at the target concentrations of 2.0, 5.0 and 8.0 μg/ml in spiked urine samples. Coefficients of variation of the recoveries ranged from 2.1 to 11.3% at these concentrations. Quantitation limits of the technique were approximately 300 ng/ml. The assay

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is highly specific for these compounds and requires a short sample preparation procedure prior to the electrophoretic analysis

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J Chromatogr A 2009 **1216** (9) 1515
Use of multiple-reaction monitoring ratios for identifying imcompletely resolved fentanyl homologs and anologs *via* ultra-high-pressure liquid chromatography-tandem mass spectrometry
Fentanyl and 16 of its corresponding homologs and analogs were identified by employing ultra-high-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). A 1.7 µm Acquity BEH C18 column (150 mm x 2.1 mm) was utilised with a 1% formic acid (pH 2.2), methanol gradient. Multiple-reaction monitoring (MRM) was used for MS/MS detection. All selected fentanyl-related compounds, including incompletely resolved compounds, were uniquely identified using retention times and dual MRMs

12 Forensics

Belal T, Awad T, DeRuiter J, Clark CR*// Auburn Univ, Harrison Sch Pharm, Dept Pharmacal Sci, 3306B Walker Bldg, Auburn, Al 36849, USA
Forensic Sci Int 2009 **184** (1-3) 54
GC-IRD methods for the identification of isomeric ethoxyphenethylamines and methoxymethcathinones
Gas chromatography with vapor phase infrared spectrophotometric detection was employed to analyse a series of 12 isomeric phenethylamines. For each of these unique isomers, the major mass spectral fragments occur at equivalent mass and all have equal molecular weight. Identification of any one of these amines and the exclusion of all other isomers was achieved with their infrared spectra. There was no need for chemical derivatization to achieve their differentiation. The methoxymethcathinones show unique infrared absorption bands in the 1690-1700/cm range for the carbonyl group and the ring substitution pattern in the ethoxymethamphetamines can be differentiated by several bands in the 700-1610/cm region. Side chain and degree of nitrogen substitution may be achieved in the 2770-3000/cm region of the infrared range. All the studied regioisomers could be distinguished from 3,4-MDMA *via* their vapor phase IR spectra. Capillary gas chromatography on an Rxi-50 stationary phase successfully resolved the side chain regioisomers, the substituted methamphetamines and the methoxymethcathinones

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Talanta 2009 **78** (1) 242
An assay for identification and determination of toxic rodenticide valone in serum by ion chromatography-electrospray ionization tandem mass spectrometry with ion trap detector
Valone has a chronic and toxic anticoagulant rodenticide. It has been widely used in China and in recent years, has resulted in some accidental and intentional intoxications. To date, a sensitive and selective method for the confirmation of valone has not been described. Therefore, the aim of this research was to establish a novel assay for the identification and quantification of valone in serum by ion chromatography-electrospray ionization tandem mass spectrometry (IC-MS/MS). Serum samples were extracted with methanol/acetonitrile (10:90, v/v) and cleaned by Oasis HLB solid-phase extraction cartridge, chromatographic separation was performed on an Ionpac AS11 column with an eluent of methanol/30 mmol/l KOH (10:90, v/v). Overall extraction efficiency was >81.0% and the limit of quantification was 0.5 ng/ml. Regression analysis of the calibration data indicated good correlation (r^2>0.99) for valone. Intra- and inter-day precisions for quality-control samples were less than 8.0 and 13.7%, respectively. This novel technique facilitates the identification and quantification of valone in both clinical and forensic specimens

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J Anal Toxicol 2009 **33** (2) 65
Enantioselective analysis of citalopram and escitalopram in postmortem blood together with genotyping for CYP2D6 and CYP2C19
Whereas citalopram is marketed as a racemate (50:50) mixture of the *S*(+)-enantiomer and *R*(-)-enantiomer, it is the active *S*(+)-enantiomer (escitalopram) that produces inhibitory effects. Escitalopram was introduced in 2002 onto the Swedish market for treatment of depression and anxiety disorders following the introduction of citalopram in 1992. The main aim of this investigation was to determine *S*(+)-citalopram [i.e., the racemic drug (citalopram) or the enantiomer (escitalopram)] present in forensic autopsy cases positive for the presence of citalopram in routine screening using a non-enantioselective bioanalytical method. Fifty out of the 270 samples determined as positive by gas chromatography-nitrogen-phosphorus detection were further analyzed using enantioselective high-performance liquid chromatography. CYP2D6 and CYP2C19 isoenzymes are implicated in the metabolism of citalopram and escitalopram. Therefore, the 50 cases were genotyped. In samples positive for racemic citalopram employing the screening method for forensic autopsy cases, up to 20% may have been misinterpreted in the absence of an enantioselective method. Therefore, an enantioselective method is required for correct interpretation of autopsy cases, after the enantiomer has been introduced onto the market. Poor metabolizers were 6% for CYP2D6 and 8% for CYP2C19

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Forensic Sci Int 2009 **185** (1-3) 100
Detection of acute fentanyl exposure in fresh and decomposed skeletal tissues
Automated enzyme-linked immunosorbent assay (ELISA) was utilised to analyse fresh and decomposed skeletal tissues (marrow and bone) for acute fentanyl exposure. Fentanyl was administered acutely to rats (n=15) at a dose of 0 (n=3), 15 (n=3), 30 (n=3) or 60 µg/kg (n=6) by intraperitoneal injection, and euthanized within 20 min. Femora and tibiae were extracted from the fresh corpses and marrow was isolated from the femoral and tibial medullary cavities. Subsequently, remains were allowed to decompose outdoors to the point of complete skeletonization, and vertebrae and pelvi were recovered for analysis. In all instances, bones were cleaned in alkaline solution and then ground into a fine powder. Marrow was homogenized in alkaline solution. Fentanyl was extracted from ground bone with methanolic solution. Extracts were adjusted to pH 6 and analyzed by ELISA. Perimortem heart blood was also obtained and diluted in phosphate buffer before to screening by ELISA. The influence of tissue type on ELISA response was examined through determination of binary classification test sensitivity and the relative decrease in absorbance (%DA, drug-positive tissues *vs* drug-free controls) in each tissue type. Overall, the %DA varied significantly between extracts from different skeletal tissues for a given dose, according to the general order of marrow>vertebrae approximately pelvi>epiphyseal bone approximately diaphyseal bone. Binary classification test sensitivity values for fentanyl in marrow, fresh epiphyseal (femoral and tibial) bone, fresh diaphyseal (femoral and tibial) bone, decomposed vertebrae and decomposed pelvic bone were 100%, 16-33%, 0-16%, 0-33% and 66-100%, respectively, at the 60 µg/kg dose level. Whereas mean %DA values showed a strong positive correlation with those in marrow and blood measurements and the administered dose (r=0.997 and 0.986), such a correlation was not noted in assays of decomposed tissues (r=-0.157 and -0.315). These results indicate that the type of skeletal tissue sampled and position within a given bone may be important considerations in the choice of substrate for fentanyl screening in skeletal tissues. In addition, quantitative analysis of drugs in decomposed bones may be of limited interpretive value

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J Anal Toxicol 2009 **33** (1) 56
Confirmation of gelsemium poisoning by targeted analysis of toxic gelsemium alkaloids in urine
Gelsemium plants are highly poisonous but toxicological evaluation of suspected poisoning cases has been hindered by the chemical complexity of the toxins involved. The collective detection of gelsemine and related alkaloids from *Gelsemium elegans* was achieved with a novel liquid chromatography-tandem mass spectrometry technique. Unexplained intoxications following the ingestion of seemingly nontoxic herbs were investigated. In three clusters of toxicological emergencies ranging from severe dizziness to respiratory failure, *Gelsemium elegans* mistaken for various look-alike therapeutic herbs was suspected to be the hidden cause of poisoning. Nine cases of gelsemium poisonings were determined by urine alkaloid profiles. Gelsemine was identified as the main urinary marker of *Gelsemium* exposure

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Forensic Sci Int 2009 **184** (1-3) 69
Use of fission yeast heterologously expressing human cytochrome P450 2B6 in biotechnological synthesis of the designer drug metabolite *N*-(1-phenylcyclohexyl)-2-hydroxyethanamine
Metabolite standards are rarely commercially available, particularly in respect of of new designer drugs. Chemical synthesis is often unwieldy. However, human cytochrome P450 (CYP) isoenzymes heterologously expressed in the fission yeast *Schizosaccharomyces pombe* may be employed for the biotechnological synthesis of drug metabolites. This technique was employed to the

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production of *N*-(1-phenylcyclohexyl)-2-hydroxyethanamine (PCHEA), the common *O*-dealkyl metabolite of the designer drugs *N*-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA) and *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA). After adding 250 µmol PCEEA x HCl (62 mg), a 1 litre culture of CAD65 (*S. pombe* strain co-expressing human CYP reductase and CYP2B6) was fermented over 65 h (pH 8, 30ºC) and centrifuged. PCHEA and non-metabolised parent drug were isolated from the supernatant by solid-phase extraction (SPE). The eluate was evaporated to dryness and reconstituted in HPLC solvent. Aliquots were separated by semi-preparative HPLC. PCHEA was extracted from the respective fraction by liquid-liquid extraction and precipitated as hydrochloric salt. Approximately 80% of PCEEA was converted to PCHEA. The final yield of PCHEA x HCl was 9 mg (35 µmol) and its identity was confirmed by GC-MS, ^{1}H NMR and ^{13}C NMR. The product purity, was determined by HPLC-UV at 95%

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Forensic Sci Int 2009 **184** (1-3) 74

Blood concentrations of clonazepam and 7-aminoclonazepam in forensic cases in Denmark for the period 2002-2007

Clonazepam is a benzodiazepine prescription drug used to treat epilepsy and anxiety. Furthermore, it is frequently employed to treat drug addicts and is a popular drug of abuse. The incidence and blood concentrations of clonazepam and its metabolite 7-aminoclonazepam in cases referred to the Section of Forensic Chemistry at the University of Copenhagen in 2002-2007 was analysed. Clonazepam was detected by LC-MS/MS in 297 traffic cases, 92 criminal cases (perpetrators or victims of a crime) and in 140 postmortem cases. The concentration ranges of clonazepam+7-aminoclonazepam were 0.002-0.840 mg/kg (median 0.067) for traffic cases, 0.005-0.913 (median 0.071) for criminal cases (offenders), 0.002-0.720 (median 0.030) for criminal cases (victims) and 0.002-1.676 (median 0.115) for postmortem cases. Concentrations were similar among the different groups but the median value was highest in the postmortem group. Usually, other drugs were also present. In the postmortem group, cases (*n*=27) with relatively high (>0.2 mg/kg) clonazepam+7-aminoclonazepam values were examined in greater detail. Other drugs were noted in all instances but clonazepam was judged to be the primary cause of death in five cases. The range of clonazepam+7-aminoclonazepam concentrations in these five cases ranged from 0.26 to 0.54 mg/kg (median 0.29 mg/kg)

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J Anal Toxicol 2009 **33** (1) 15

Postmortem tissue concentrations of olanzapine

Twenty-eight cases from the San Diego County Medical Examiner's Office during the period 2004-2007 involving olanzapine were analyzed. Initially, it was detected by gas chromatography coupled with mass spectrometry (GC-MS), and then confirmed by GC with a nitrogen-phosphorus detector. Concentrations in peripheral blood (PB), central blood (CB), liver, and vitreous were determined where available. Average olanzapine concentrations in the six olanzapine-only deaths (mean+/-standard deviation) were 3.2+/-2.0 mg/l (PB), 4.5+/-2.6 mg/l (CB), 40+/-29 mg/kg (liver), and 1.6+/-0.50 mg/l (vitreous). In the 10 non-olanzapine-related deaths, average olanzapine concentrations were 0.26+/-0.13 mg/l (PB), 0.29+/-0.17 mg/l (CB), 5.6+/-5.6 mg/kg (liver), and 0.24+/-0.38 mg/l (vitreous). For the 10 multi-drug deaths, average concentrations were 0.59+/-0.33 mg/l (PB), 0.64+/-0.60 mg/l (CB), 5.9+/-4.3 mg/kg (liver), and 0.78+/-0.91 mg/l (vitreous). Concentrations of olanzapine associated with toxicity were found to be in the range of 1.4-6.2 mg/l (PB), 1.1-7.4 mg/l (CB), 14-88 mg/kg (liver), and 1.1-2.1 mg/l (vitreous). Concentrations associated with therapeutic use were found to be in the range of 0.11-0.43 mg/l (PB), 0-0.53 mg/l (CB), 0-8.6 mg/kg (liver), and 0-0.98 mg/l (vitreous). Deaths resulting solely from olanzapine were identified by a 10-fold or more increase in tissue concentrations greater than those found in the non-olanzapine-related deaths

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J Chromatogr B 2009 **877** (7) 615

Optimisation of HPLC gradient separations using artificial neural networks (ANNs): Application to benzodiazepines in post-mortem samples

Separation of nine benzodiazepines was achieved using artificial neural networks (ANNs) in conjunction with an experimental design to optimise gradient HPLC. Using the most promising ANN, the optimum conditions predicted were 25 mM formate buffer (pH 2.8), 10% MeOH, acetonitrile (ACN) gradient 0-15 min, 6.5-48.5%. For six of the nine analytes, the error associated with the prediction of retention times and peak widths under these conditions was less than 5%. The optimised method, with limits of detection (LODs) in the range of 0.0057-0.023 µg/ml and recoveries between 58% and 92%, was successfully applied to genuine post-mortem samples. This technique represents a more flexible and convenient means for optimising gradient elution separations using ANNs than has been reported previously.

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J Anal Toxicol 2009 **33** (2) 85

High-performance liquid chromatographic determination of chlorhexidine in whole blood by solid-phase extraction and kinetics following an intravenous infusion in rats

Extraction and analysis of chlorhexidine (CHX) from whole blood using solid-phase extraction (SPE) together with high-performance liquid chromatography (HPLC) is described. Samples of blood were spiked with chlorpromazine as an internal standard, were fortified with sodium acetate buffer and purified with Bakerbond C_{18} SPE columns. Columns were washed, dried, and eluted with experimental optimized solvent systems. HPLC employed a Capcell Pak C_{18} MG column (4.6 x 250-mm) and monitored at 260 nm, using a UV detector. A mobile phase consisting of acetonitrile/water (40:60 v/v), containing 0.05% trifluoroacetic acid, 0.05% heptafluorobutyric acid, and 0.1% triethylamine, was utilised. Over the range of 0.05 to 2.0 µg/g, the assay was linear and the limit of detection was 0.01 µg/g for CHX in whole blood. In the concentration range of 0.05 to 2.0 µg/g, the recoveries were from 72% to 85%, and the intra- and interday precision, expressed as coefficient of variation, were less than 11% and 13%, respectively. By employing the above method, kinetic characteristics following an intravenous infusion of a CHX product, Maskin solution, at a dose of 15 mg/kg in rats were determined. The kinetic profiles of CHX conformed to a two-compartment model with an α half-life (of distribution) at 0.05 h and a beta half-life (of elimination) at 0.55 h in rats. The above technique is simple and reliable. It may be employed for the determination of CHX in blood samples and may applied to forensic and clinical specimens

13 Alcohol

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J Anal Toxicol 2009 **33** (1) 27

Quantitative determination of caffeine and alcohol in energy drinks and the potential to produce positive transdermal alcohol concentrations in human subjects

Positive "alcohol alerts" based on transdermal alcohol concentration (TAC) using a commercially available electrochemical monitoring device resulting from non-alcoholic energy drinks were studied. Ethanol and caffeine were quantitatively assayed in eleven energy drinks. Ethanol concentrations for all of the non-alcoholic energy drinks ranged in concentration from 0.03 to 0.230% (w/v) and caffeine content per 8-oz serving ranged from 65 to 126 mg. Fifteen human subjects consumed between 6 and 8 energy drinks over an 8-h period. The SCRAM II monitoring device was used to analyse TACs every 30 min before, during, and after the study. However, none produced TAC readings indicative of a positive "alcohol alert". TAC measurements for all subjects before, during and after the energy drink study period (16 h total) were <0.02% (w/v). The quantity of non-alcoholic energy drink consumed greatly exceeded what might be considered typical. Therefore, it would appear that energy drink consumption is an unlikely explanation for elevated TACs that might be identified as potential drinking episodes or "alcohol alerts" using this device

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J Anal Toxicol 2009 **33** (2) 109

Assessment of response of the Intoxilyzer 8000C to volatiles of forensic relevance *in vitro*, part I: Acetone, isopropanol, and methanol

The Intoxilyzer 8000C (version approved for evidentiary breath alcohol testing in Canada) was evaluated for response to volatile solvents *in vitro*. Acetone, isopropanol, and methanol were prepared as aqueous solutions or dilutions of standard alcohol solution (SAS; 1.21 mg ethanol/ml) to produce equivalent blood ethanol concentrations (aBEC) of 50 or 80 mg/dl. Solvent concentrations employed were relevant to clinical or impaired driving scenarios. Replicates of 20 aBEC measurements were made for each mixture and the actuation of the "INTERFERANT DETECT" message (IDM) was noted. Measurements of aqueous acetone (0-40 mg acetone/dl), isopropanol (0-100 mg isopropanol/dl), and methanol (0-100 mg methanol/dl) yielded aBECs of 0, 0-43, and 0-55 mg/dl, respectively. The minimum concentration examined at which the IDM was actuated in 100% of replicates was 25, 30, and 100 mg/dL for acetone, isopropanol, and methanol, respectively. Whereas, the maximum concentration

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examined at which the IDM was actuated in none of the replicates was 5, 10, and 50 mg/dl for acetone, isopropanol, and methanol, respectively. Investigations of acetone/isopropanol mixtures in diluted SAS where the IDM was not always actuated, demonstrated the maximum BEC overestimation at 10 mg/dl. Consequently, the potential for significant undetected BEC overestimation is low and may be further reduced through truncation of test results and subject observation

14 Tobacco

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J Agric Food Chem 2009 **57** (7) 2678

Gas/particle partitioning of two acid-base active compounds in mainstream tobacco smoke: Nicotine and ammonia

In mainstream tobacco smoke (MTS) for a selection of cigarettes, "little cigars", and biddies, gas/particle (G/P) partitioning constant (K_p) values are calculated for nicotine and ammonia. There is an increase in volatility of nicotine from the smoke particulate matter as K_p^{nic} decreases as a result of the increasing basicity in the MTS. "Little cigars" and biddies produced generally lower Kp^{nic} values and higher unbound ammonia levels than most of the cigarettes, indicating a correlation between the two parameters. However, solely within the cigarettes, there was little correlation. The water content of MTS particulate matter was found to influence both K_p^{nic} and K_p^{amm}. Unbound ammonia is actual NH_3/NH_4^+; bound ammonia is comprised of compounds such as amides of ammonia; total ammonia is unbound + bound. Previous studies of ammonia in MTS have frequently not accurately measured either unbound or total ammonia: the acidic solutions formerly used to determine ammonia in MTS produce ammonia from bound forms by hydrolysis, and the release in those studies may not have been complete. This study suggests that a thorough examination of unbound and bound ammonia in MTS is required before the role of ammonia in affecting volatility of nicotine in MTS may be understood

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J Pharm Biomed Anal 2009 **49** (1) 108

Determination of nicotine, cotinine, and related alkaloids in human urine and saliva by automated in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry

A simple, rapid and sensitive technique for the determination of nicotine, cotinine, nornicotine, anabasine, and anatabine in human urine and saliva has been produced. On-line in-tube solid-phase microextraction (SPME) coupled with liquid chromatography-mass spectrometry (LC-MS) was employed. Separations within 7 min for nicotine, cotinine and related alkaloids were achieved with high performance liquid chromatography (HPLC) using a Synergi 4u POLAR-RP 80A column and 5 mM ammonium formate/methanol (55/45, v/v) as a mobile phase at a flow-rate of 0.8 ml/min. Electrospray ionization conditions in the positive ion mode were optimized for MS detection. Optimum in-tube SPME conditions were 25 draw/eject cycles with a sample size of 40 µl using a CP-Pora PLOT amine capillary column as the extraction device. Extracted compounds could be desorbed easily from the capillary by passage of the mobile phase, and no carryover was noted. Employing the in-tube SPME LC-MS method, the calibration curves were linear in the concentration range of 0.5-20 ng/ml of nicotine, cotinine and related compounds in urine and saliva, and the detection limits (S/N=3) were 15-40 pg/ml. The technique described here demonstrated a 20-46-fold higher sensitivity than the direct injection method (5 µl injection). Within-run and between-day precision (relative standard deviations) were below 4.7% and 11.3% (n=5), respectively. Urine and saliva samples were successfully analysed without interference peaks. Nicotine, cotinine and related compounds spiked into urine and saliva samples were recovered above 83%, and the relative standard deviations were below 7.1%. Urinary and salivary levels of these compounds in nicotine intake and smoking were analysed

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J Chromatogr B 2009 **877** (3) 339

Determination of hair nicotine by gas chromatography-mass spectrometry

Long-term environmental tobacco smoke (ETS) exposure and smoking status may be monitored *via* hair nicotine. Usually, hair nicotine analysis involves alkaline digestion, extraction and instrumental analysis. A gas chromatography-mass spectrometry (GC-MS) method was shown to be of high throughput with average approximately 100 hair samples being extracted and analyzed per day. Simplified extraction procedure and shortened GC analysis time facilitated throughput. Extraction was improved with a small volume (0.4 ml) of organic solvent that did not require further evaporation and salting steps before to GC-MS analysis. The amount of hair utilized in the extraction was quite small (5 mg). Sensitivity and selectivity of the assay wass equal, if not better than other established methods. The linearity of the assay ($r^2 > 0.995$), limit of quantitation (0.04 ng/mg hair), within- and between-assays accuracies and precisions (<11.4%) and mean recovery (92.6%) were within the acceptable range

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J Chromatogr A 2009 **1216** (12) 2227

Development of an improved method for the determination of polycyclic aromatic hydrocarbons in mainstream tobacco smoke

The quantitation of 16 polycyclic aromatic hydrocarbons found in mainstream tobacco smoke condensate has been analysed by a gas chromatograph/mass-selective detection (GC/MS) technique. Significant reductions in analysis run times and increased accuracy were achieved by the utilization of two types of solid-phase extraction media combined with capillary column technology which also removed matrix interferences. A chilled impinger was employed to trap semi-volatile polycyclic aromatic hydrocarbons and to provide more accurate data. This was achieved without sacrificing the repeatability, reproducibility, and precision obtained in previously published methods. An improved, robust analytical method capable of increasing laboratory capacity and reducing sample reporting time has been developed and validated

15 Homeland Security

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Food Agric Immunol 2009 **20** (1) 11

Development of monoclonal antibodies specific for *Ricinus* agglutinins

The castor oil bean (*Ricinus communis*) is grown principally as a source of high quality industrial lubricant and as an ornamental. However, it contains ricin, a highly toxic, dichain ribosome-inactivating protein in the seeds. Its presence in industrial byproducts and its documented use for intentional poisoning necessitates analytical techniques to quantify ricin in both castor extracts and food matrices. A panel of monoclonal antibodies to ricin has been produced, with most having strong cross-reactivity with RCA-1, a homologous but less toxic castor agglutinin. Some of the IgM-producing hybridomas appeared to produce a second, IgG isotype and were further analysed by fluorescence-activated cell sorting. The antibodies were effective in various ELISA formats, many with IC50's in the range of 0.1-10 ng/ml and minimal matrix effects in skim milk. Assay specificity may be modified to suit analytical requirements by varying the combination of antibodies in a sandwich ELISA format.

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Anal Chem 2009 **81** (4) 1529

Detection of intact ricin in crude and purified extracts from castor beans using matrix-assisted laser desorption ionization mass spectrometry

Seeds of the castor bean plant contain the lectin ricin which is a highly toxic protein. Crude extracts from castor beans may be toxic by several routes. The use of these extracts by terrorist organizations has resulted in international concern. The rapid detection of this lectin in air samples is critical in determining the illicit use of this material due to its lethality in aerosolized form. Matrix-assisted laser desorption ionization (MALDI) mass measurement with an automated laser firing sequence was emplyed to detect intact ricin from solutions containing less than 4 µg/ml of ricin in the presence of other endogenous seed proteins. Sensitivity was improved by the addition of 0.01% Tween 80 to the extracts which resulted in a greatly enhanced ricin signal. Moreover, this treatment substantially reduced the interference from castor bean seed storage proteins. Frequently, the ricin signal may be completely obscured by oligomers of seed storage proteins. However, this treatment reveals the ricin molecular ion, facilitating a judgment as to the ricin content of the extract. This technique allows sensitive and rapid identification of intact ricin from aqueous samples with little sample preparation and is amenable to automatic acquisition

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J Food Prot 2009 **72** (4) 903

Application of deadenylase electrochemiluminescence assay for ricin to foods in a plate format

Based on the catalytic activity of the toxin subunit A chain, a recently developed bead-based deadenylase electrochemiluminescence assay for ricin which

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is simple and sensitive has been modified to work in a 96-well plate format. The technique was evaluated by using juice samples. Unlike the bead-based assay, the plate-based assay includes wash steps that enable the removal of food particles which minimizes matrix effects and improves the signal-to-noise ratios and limits of detection (LOD). The LOD values for ricin in apple juice, vegetable juice, and citrate buffer by using the bead-based assay were 0.4, 1, and 0.1 µg/ml, respectively. In contrast, the LOD values for ricin by using the plate-based assay were 0.04, 0.1, and 0.04 µg/ml in apple juice, vegetable juice, and citrate buffer, respectively. Therefore, the plate-based assay displayed three- to 10-fold lower LOD values compared with the bead-based assay. Signal-to-noise ratios for the plate-based assay were similar to those for the bead-based assay for ricin in citrate buffer, but 2- to 4.5-fold higher when the plate-based assay was employed for analysis of juice samples

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J Anal Toxicol 2009 **33** (2) 77

Quantification of L-abrine in human and rat urine: A biomarker for the toxin abrin

The jequirity seed contains a toxic protein known as abrin. L-Abrine (*N*-methyl-tryptophan) may be employed as a biomarker for abrin exposure. Analysis of L-abrine was added to an existing method for quantifying ricinine as a marker for ricin exposure in human urine and analytically validated. Accuracy and reproducibility were improved by addition of a newly synthesized $^{13}C_1{}^{2}H_3$-L-abrine internal standard. Samples of urine (1ml) were analysed using solid-phase extraction prior to a 6-min high-performance liquid chromatography separation. Protonated molecular ions were formed *via* electrospray ionization in a triple-quadrupole mass spectrometer and quantified *via* multiple reaction monitoring. Quality control materials involved two enriched urine pools which were used for method validation. Endogenous levels of L-abrine were quantified in a reference range of 113 random urine samples at 0.72 +/- 0.51 ng/ml. Concentrations of L-abrine in urine were monitored in an intentional rat exposure study for up to 48 h. A comparison of the results from the human reference range and the animal exposure study indicates that this method is suitable for quantifying L-abrine within 24 h post-exposure. After 24 h, quantification of L-abrine is limited by rapid excretion of the biomarker and the level of the L-abrine dose

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Anal Chem 2009 **81** (6) 2037

Mass spectrometric detection of ricin and its activity in food and clinical samples

Ricin is a potent lectin toxin which inhibits protein synthesis and may cause death or respiratory failure. Ricin is considered a likely agent for bioterrorism due to its ready availability and lethality. Therefore, an important public health goal is a technique for the rapid determination of food and human exposure. Herein is described the development of a technique for the detection of ricin and its activity in food or clinical samples. The technique comprises immunocapture of the toxin, examination of the activity of the ricin protein upon a DNA substrate that mimics the toxin's natural RNA target, and analysis of tryptic fragments of the toxin itself. The combination of these three techniques on the same sample allows a sensitive and selective analysis of ricin isolated from a food or clinical samples. The analysis includes a measure of the toxin's activity. The utility of this method was illustrated by analysis of ricin spiked into food and clinical samples consisting of milk, apple juice, serum, and saliva

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Anal Bioanal Chem 2009 **393** (8) 1949

Screening hydrolysis products of sulfur mustard agents by high-performance liquid chromatography with inductively coupled plasma mass spectrometry detection

The class of mustard agents includes sulfur mustard (HD), bis(2-chloroethyl)sulfide which may be deployed in chemical warfare. When sulfur mustards degrade the main chemical warfare hydrolysis products include thiodiglycol, bis(2-hydroxyethylthio)methane, 1,2-bis(2-hydroxyethylthio)ethane, 1,3-bis(2-hydroxyethylthio)propane, and 1,4-bis(2-hydroxyethylthio)butane. These five hydrolysis degradation products were determined by employing reversed-phase high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (ICP-MS) for element-specific sulfur detection using a collision/reaction cell and electrospray ionization mass spectrometry to confirm the identification. This is the first investigation employing ICP-MS with ^{32}S element-specific detection for the analysis of vesicant chemical warfare agent degradation products

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Anal Chim Acta 2009 **634** (2) 255

A capactive immunosensor for detection of cholera toxin

Biological toxins both as contaminants of food and their potential use as weapons of mass destruction has resulted in an urgency for rapid and cost effective analytical techniques capable of detecting them in trace amounts. A sensitive method for detection of cholera toxin (CT) using a flow-injection capacitive immunosensor based on self-assembled monolayers is described. The surface of the sensor employs monoclonal antibodies against the B subunit of CT (anti-CT) immobilized on a gold transducer. The immunosensor responded linearly to CT concentrations in the range from $1.0x10^{-13}$ to $1.0x10^{-10}$ M under optimized conditions. The limit of detection (LOD) was $1.0x10^{-14}$ M. CT was detected by two analytical techniques employing the same antibody; sandwich ELISA and surface plasmon resonance (SPR)-based immunosensor. The former had an LOD of $1.2x10^{-12}$ M and a working range from $3.7x10^{-11}$ to $2.9x10^{-10}$ M whereas, the later had an LOD of $1.0x10^{-11}$ M and a linearity ranging from $1.0x10^{-9}$ to $1.0x10^{-6}$ M. Therefore, the capacitive immunosensor system had a higher sensitivity than the other two techniques. The binding affinity of CT to the immobilized anti-CT was determined using the SPR-based immunosensor and an association constant (K_A) of $1.4x10^{9}$ M was calculated

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Sensor Actuator B Chem 2009 **135** (2) 399

Development of handheld SAW vapor sensors for explosives and CW agents

Discrete (single sensor) surface acoustic wave (SAW) handheld sensor systems have been produced for the detection and quantification of explosives and chemical warfare agents (CWA). The sensing element employed was a SAW device of delay line, resonator or dispersive delay line type with frequency of operation ranging from 36 MHz to 434 MHz. Various polymers having good selectivity to explosives and CWA were used to coat the SAW devices. Oscillator with the SAW device in the feedback loop as a frequency determining element was utilised. Dual oscillator configuration with one coated and one uncoated SAW device was employed. The outputs of the oscillators were mixed and signal conditioned before frequency measurement. A high-speed high-resolution reciprocal counting method using microcontroller-based readout circuitry was employed for frequency measurement. Online display and storage of data employed acquisition software written in Visual Basic. The stability, resolution, accuracy and sensitivity was analyzed in respect of the performance of various parts and the sensor systems

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Anal Chem 2009 **81** (7) 2477

Fragmentation energy index for universalization of fragmentation energy in ion trap mass spectrometers for the analysis of chemical weapon convention related chemicals by atmospheric pressure ionization-tandem mass spectrometry analysis

The production of searchable library databases has been facilitated by the use of mass spectra generated at 70 eV in electron ionization (EI) as a universal standard. It has had a major influence on the analytical applications of gas chromatography/mass spectrometry (GC/MS) and for liquid chromatography tandem mass spectrometry (LC-MS/MS). This has resulted in a novel method to standardize the collisional fragmentation energy for the analysis of Chemical Weapon Convention (CWC)-related chemicals by atmospheric pressure ionization-tandem mass spectrometry (API-MS(n)) using three-dimensional (3D) ion trap instruments. A "fragmentation energy index" (FEI) is proposed for normalizing fragmentation energy. This is an arbitrary scale based on specific MS/MS fragmentation obtained at different collisional energies for reference chemicals which are not CWC scheduled compounds. FEI 6 for the generation of an MS^n library-searchable mass spectral database is recommended

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Bull Korean Chem Soc 2009 **30** (1) 49

Analysis of chemical warfare agents in water using single-drop microextraction

Single-drop microextraction (SDME) was evaluated for the GC-MS determination of one class of the chemical warfare agents (CWAs), namely, nerve agents. It is important to detect the nerve agents in the environmental samples because of their acute toxicity. Several affecting parameters including extraction solvents, stirring rate, extraction time, and amounts of salt were optimized. The limit of detections (LODs) were 0.1 - 10 ng/ml and the relative standard deviations (RSDs%, n=5) were in the range of 6.3% to 9.0% for four nerve

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agents. Without pretreatment of the environmental samples, 5-103 fold enrichments and 48-100% recovery were achieved. These results demonstrate the feasibility of this method for on-site and off-site analysis of water samples collected from suspicious CWAs sites

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Clin Vaccine Immunol 2009 **16** (3) 408

Detection of anthrax toxin by an ultrasensitive immunoassay using europium nanoparticles

A europium nanoparticle-based immunoassay (ENIA) for the sensitive detection of anthrax protective antigen (PA) has been developed. The ENIA produced a linear dose-dependent pattern within the detection range of 0.01 to 100 ng/ml. In addition, it was approximately 100-fold more sensitive than enzyme-linked immunosorbent assay (ELISA). Employing serum samples from healthy adults, mouse plasma without PA, or plasma samples collected from mice injected with anthrax lethal factor or edema factor alone false-positive results were not obtained. When plasma samples were spiked with PA, the detection sensitivities for ENIA and ELISA were 100% (11/11 samples) and 36.4% (4/11 samples), respectively. The assay resulted in a linear but qualitative correlation between the PA injected and the PA detected in murine blood (r=0.97731; P<0.0001). In addition, anthrax PA was detected in the circulation of mice infected with spores from a toxigenic Sterne-like strain of *Bacillus anthracis*, However, detection was only possible in the later stages of infection. The universal labeling technology based on europium nanoparticles and its application to provide a rapid and sensitive testing platform for clinical diagnosis and laboratory research is demonstrated

Wang DB, Bi LJ, Zhang ZP, Chen YY, Yang RF, Wei HP, Zhou YF, Zhang XE*// *Chinese Acad Sci, Wuhan Inst Virology, State Key Laboratory Virology, CN-430071 Wuhan, Peoples Rep China

Analyst 2009 **134** (4) 738

Label-free detection of *B. anthracis* spores using a surface plasmon resonance biosensor

For the first time, the use of surface plasmon resonance (SPR) has been employed for the rapid, sensitive and label-free detection of whole *B. anthracis* spores. This technique utilises an SPR biosensor (Biacore 3000), and a monoclonal antibody which was raised against the *B. anthracis* spore (mAb 8G3). Employing subtractive inhibition assays, entire *B. anthracis* spores with concentrations as low as 10^4 colony-forming units (CFU)/ml may be detected within 40 min. Even in high concentrations, other related *Bacillus* spores, may be differentiated from *B. anthracis* spores

16 Workplace

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Anal Biochem 2009 **386** (2) 186

An approach based on liquid chromatography/electrospray ionization-mass spectrometry to detect diol metabolites as biomarkers of exposure to styrene and 1,3-butadiene

Styrene and 1,3-butadiene are important compounds extensively employed in the plastics industry. Cytochrome *P*450-mediated oxidation results in the corresponding epoxides, which are subsequently converted to diols by epoxide hydrolase or through spontaneous hydration. Consequently, styrene glycol and 3-butene-1,2-diol have been suggested as biomarkers of exposure to styrene and 1,3-butadiene, respectively. However, poor ionization of the diols within electrospray mass spectrometers becomes an issue in the detection of the two diols by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS). An LC/ESI-MS approach has been developed to analyze styrene glycol and 3-butene-1,2-diol utilising derivatization with 2-bromopyridine-5-boronic acid (BPBA). This not only results in dramatic increases of the sensitivity of diol detection but also facilitates the identification of the diols. This analytical technique was simple, quick, and convincing without the need for complicated chemical derivatization. To investigate the feasibility of BPBA as a derivatizing reagent of diols, the impact of their configuration on the affinity of a selection of diols to BPBA employed the established LC/ESI-MS approach. Both cis and trans diols may be derivatized by BPBA. Therefore, BPBA may be employed as a general derivatizing reagent for the detection of vicinal diols by LC/MS

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Microchem J 2009 **91** (2) 149

Determination of fentanyl in human breath by solid-phase microextraction and gas chromatography-mass spectrometry

Fentanyl, a type of intravenous narcotic analgesic, is commonly employed in clinical anesthesia. It was detected in both the air of the cardiothoracic operating room and patients' expiratory circuit. However, whether the fentanyl in patients' expiratory circuit is exhaled by is unknown. Breath samples were obtained from the expiratory circuits of anesthetic equipment linked to patients who received intravenous fentanyl. Solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS) was developed to detect and quantify fentanyl in breath samples. The factors influencing adsorption (extraction time, temperature,) and desorption (desorption time) of the fentanyl on the fiber were investigated and validated for method development. The technique proved to be simple, easy, and inexpensive and offer high sensitivity and reproducibility. Linear range was obtained from 0.05 ng/ml to 0.8 ng/ml. The limit of detection was 0.01 ng/ml while an interday precision of less than 12.13% (n = 5) could be achieved. Six patients were involved in this study. Analysis demonstrated the presence of fentanyl in the breath of patients who received intravenous fentanyl, and fentanyl concentrations in breath varied from 6.00 to 20.89 pg/ml thereby confirming that it may be exhaled by patients who received intravenous fentanyl.

17 Product Authenticity

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Spectrosc Eur 2009 **21** (3) 10

DOSY NMR, a new tool for fake drug analyses

Abstract not available

Gryniewicz CM, Reepmeyer JC, Kauffman JF, Buhse LF// US/FDA, Div Pharmaceut Analysis, 1114 Market St, St Louis, Mo 63101, USA

J Pharm Biomed Anal 2009 **49** (3) 601

Detection of undeclared erectile dysfunction drugs and analogues in dietary supplements by ion mobility spectrometry

The presence of undeclared synthetic erectile dysfunction (ED) drugs or drug analogues in herbal dietary supplements claiming to enhance male sexual performance were screened by employing an ion mobility spectrometry (IMS) technique. Ion mobility spectra of authenticated reference materials including three FDA approved drugs (sildenafil citrate, tadalafil, vardenafil hydrochloride trihydrate) and five previously identified synthetic analogues (methisosildenafil, homosildenafil, piperidenafil, thiosildenafil, thiomethisosildenafil) were analysed to determine their reduced ion mobilities (K_0). All eight compounds exhibited reduced mobilities between 0.8257 and 1.2876 $cm^2/(Vs)$. Subsequently, 26 herbal products were screened for the presence of these compounds. Fifteen of the 26 products tested positive for the presence of ED drug or drug analogue adulterants based on their reduced ion mobilities. IMS results were compared with those produced by an independent LC/MS reference method for identical samples. Adulterated dietary supplements were classified with 100% accuracy and most of the undeclared drugs were correctly identified by comparison of the K_0 of the adulterant to the K_0 of the authenticated reference material. It is suggested that IMS is a viable method for testing herbal dietary supplements for the presence of ED drug or drug analogue adulterants

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Isolation and identification of hydroxythiohomosildenafil in herbal dietary supplements sold as sexual performance enhancement products

A non-declared compound was detected in herbal dietary supplements sold as sexual performance enhancement products purchased over the internet. ESI-MS/MS, NMR, UV and IR were employed to determine its structure. The compound was identified as hydroxythiohomosildenafil, an analogue of sildenafil in which the oxygen atom is substituted with a sulfur atom in the pyrazolopyrimidine moiety, and a hydroxyethyl group instead of a methyl group is attached to the piperazinyl nitrogen. This is the first report of this hydroxythiohomosildenafil being detected in herbal dietary supplements. The UV, IR and completely assigned NMR data of hydroxythiohomosildenafil were determined

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Structure elucidation of thioketone analogues of sildenafil detected as adulterants in herbal aphrodisiacs

Herbal dietary supplements marketed as aphrodisiacs were found to contain two analogues of sildenafil. Both compounds were identified as thioketone analogues of sildenafil in which the carbonyl group in the pyrimidine ring of sildenafil was substituted with a thiocarbonyl group. Thiosildenafil, a compound that has recently been reported as an adulterant in health supplements was identified as the first compound. The second compound's structure was established using LC-MS, UV spectroscopy, ESI-MS^n, NMR and a hydrolytic process. A detailed study of the hydrolysis products of sildenafil, thiosildenafil, and the second unknown compound proved that it had a structure analogous to sildenafil in which the *N*-methylpiperazine moiety had been replaced with 2,6-dimethylpiperazine and the oxygen atom of the carbonyl group in the heterocyclic ring had been replaced with a sulfur atom and was named thiomethisosildenafil. Employing the hydrolytic reaction conditions used in this study, thioketones hydrolyze to ketones (e.g., thiosildenafil→sildenafil), making this a valuable technique for the structure elucidation of thiosildenafil analogues. Ten herbal dietary supplements, each as a capsule dosage form, were discovered to contain 8-151 mg of thiomethisosildenafil per capsule, and one herbal dietary supplement was found to contain 35 mg of thiosildenafil per capsule

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Modified local straight-line screening to detect synthetic drugs in adulterated herbal medicines

Based on infrared spectroscopy, a local straight-line screening (LSLS) algorithm was recently designed as a method to detect synthetic drug(s) in adulterated herbal medicines. Herein, adjustments are made to improve the existing LSLS algorithm, including interpolation, second derivation, and change of calculation regions from 3 to 7 data points. These modifications have mitigated the effect of unpredicted noises and baseline shift on infrared spectroscopy, producing spectral characteristics of the suspected synthetic drugs in outstanding detail. The algorithm has been applied to five kinds of synthetic drugs (sibutramine, fenfluramine, lovastatin, sildenafil, and methyldopa) in 40 herbal medicine samples. The amounts of the synthetic drug(s) predicted by the modified LSLS algorithm was closer to those measured by high-performance liquid chromatography. The correct results increased from 30 obtained using the original LSLS to 36 obtained using the modified LSLS in 40 samples. The false negative responses dropped from 5 to 1, and the false positive responses dropped from 5 to 3. The results produced employing the M-LSLS algorithm based on the sibutramine spectrum collected at different times and on different instruments also varied within acceptable ranges. This technique facilitates the preliminary screening of herbal medicines suspected of adulteration with synthetic drugs, with high rapidity, accuracy, and cost effectiveness

18 Techniques

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Imprinted polymers for chiral resolution of (+/-)-ephedrine. Part 3: NMR predictions and HPLC results with alternative functional monomers

For the molecular imprinting of (-)-ephedrine, the monomers trifluoromethacrylic acid (TFMAA), 2-hydroxyethylmethacrylate (HEMA) and itaconic acid (IA) have been compared . Employing the program HypNMR, data from NMR titrations were analysed to obtain association constants for monomer-template (M-T) complexes of different stoichiometries. These were utilised to predict the speciation in imprinting mixtures with porogen and cross-linker, and molecularly imprinted polymers (MIPs) were constructed and their ability to bind (-)-ephedrine and its enantiomer were investigated with high performance liquid chromatography (HPLC). TFMAA and IA interact more strongly with ephedrine than does MAA. However, MIPs made with each of these monomers does not perform as well. Using TFMAA, covalent monomer-template adducts and TFMAA oligomers, present in the polymerisation mixture, may detract from the MIP recognition properties. Employing IA, the relative flexibility of the monomer may be an issue. HEMA interacts more weakly with ephedrine, and HEMA-based MIPs exhibit far worse retention and poorer recognition compared those based on MAA. It may be expedient to employ a higher ratio of M : T in the case of HEMA because the monomer interacts with the cross-linker EDMA

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Determination of selected -receptor antagonists in biological samples by solid-phase extraction with cholesterolic phase and LC/MS

Five selected β-receptor antagonists have been analysed by a new method involving HPLC and with emphasis on sample preparation *via* retention on a new type of silica gel sorbent used for solid-phase extraction (SPE). Chemical modification of silica gels of various porosities by cholesterol ligands were employed to produce the sorbent. Spectroscopic methods and elemental analysis were used to investigate the cholesterol-based packing material. With this extraction procedure, recoveries obtained were optimum over a relatively wide sample pH range (3.08-7.50). Sample loading, the washing step and elution conditions, the concentration of β-receptor antagonists to be extracted, and the type of sorbent were notable parameters in the sample preparation procedure. Consequently, they would therefore need to be controlled to achieve optimum recoveries of the analytes. When conditions were optimised, the recoveries of nadolol, acebutolol, esmolol, oxprenolol and propranolol from spiked buffers, blood and urine were reproducible and dependent on the polarity or hydrophilicity of the compounds. Compounds were determined by reverse-phase high-performance liquid chromatography (HPLC) with UV and ESI-ion trap mass spectrometry (MS) detection. The above technique was found to be suitable for the routine measurement of compounds that are both polar and basic, and may be employed for the analysis of biological samples such as urine and blood in clinical, toxicological or forensic laboratories. Recovery measurements were performed on spiked human urine and serum, and on real samples of mouse blood serum

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Qualitative and quantitative analysis of aconitine-type and lipo-alkaloids of *Aconitum carmichaelii* roots

A reliable and precise HPLC method coupled with photodiode array detection (HPLC-DAD) has been developed for the identification and quantification of three major aconitine-type alkaloids (aconitine, mesaconitine, hypaconitine) in the roots of *Aconitum carmichaelii* Debeaux by optimizing the extraction and analytical conditions. The qualitative analysis of the plant material was carried out by LC-APCI-MS^n. By employing this technique, 26 lipo-alkaloids were also identified from the roots of *A. carmichaelii*. The effect of processing on aconitine-type alkaloids, lipo-alkaloids and pure aconitine was investigated. As part of the study, two lipo-alkaloids, 14-benzoylaconine-8-palmitate and 14-benzoylaconine-8-linoleate were produced semisynthetically. The COX-1, COX-2 and LTB_4 formation inhibitory activity of aconite root extracts and different types of diterpene alkaloids and the toxicity of lipo-alkaloids were also examined

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Development and validation of an LC-MS/MS method for the quantification of ephedrines in urine

A simple and robust LC-MS/MS method for the quantification of ephedrine type substances in urine has been developed. Sample preparation involved a 10-fold dilution step of the samples into the internal standard solution (ephedrine-d_3, 4 µg/ml in water). Baseline separation of the diastereoisomers norpseudoephedrine-norephedrine and ephedrine-pseudoephedrine was achieved on a C_8-column employing isocratic conditions followed by positive electrospray ionisation and tandem mass spectrometric detection. The mobile phase consisted of 98/2 (H_2O/ACN) containing 0.1% HAc and 0.01% TFA. Calibration curves were produced between 2.5 and 10 µg/ml for norephedrine and norpseudoephedrine and 5 and 20 µg/ml for ephedrine, pseudoephedrine and methylephedrine. The bias ranged from -5.5 to 12% for norephedrine, -4.1 to 8.0 % for norpseudoephedrine, 0.3 to 2.1 % for ephedrine, 1.6 to 2.6 % for pseudoephedrine and 2.9 to 5.0 % for methylephedrine. Precision of the method varied between 2.8 and 10.4% for all compounds and the matrix effect was less than 15%. Forty urine samples were analysed to demostrate the applicability of the method. Results were compared with those produced using the common GC-NPD method. Results showed a good correlation between both methods with correlation coefficients higher than 0.95 for all analytes

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Preparation and evaluation of propranolol molecularly imprinted solid-phase microextraction fiber for trace analysis of -blockers in urine and plasma samples

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A novel molecularly imprinted polymer (MIP)-coated solid-phase microextraction (SPME) fiber with propranolol as template was prepared with an improved multiple co-polymerization technique. The characteristics and application of the fibers was investigated. The MIP coating was highly crosslinked and porous with the average thickness of only 25.0 μm. Therefore, the adsorption and desorption of β-blockers within the MIP coating could be rapidly performed. The specific selectivity of the MIP-coated fibers to propranolol and its structural analogues such as atenolol, pindolol, and alprenolol was examined. Non-specific adsorption could only be demonstrated with the non-imprinted polymer (NIP)-coated fibers and the extraction efficiencies of propranolol and pindolol with the MIP-coated fibers were higher markedly than that with the commercial SPME fibers. A MIP-coated SPME coupled with high-performance liquid chromatography (HPLC) method for propranolol and pindolol determination was produced under the optimized extraction conditions. Linear ranges for propranolol and pindolol were 20-1000 μg/l and detection limits were 3.8 and 6.9 μg/l, respectively. Following extraction with organic solvent, propranolol and pindolol in spiked human urine and plasma samples could be simultaneous determined with satisfactory recoveries by employing this method

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Detection of drugs and their metabolites in dusted latent fingermarks by mass spectrometry
A simple method for detecting a range of drugs within latent finger marks using surface assisted laser desorption/ionisation time-of-flight mass spectrometry (SALDI-TOF-MS) in positive ion reflectron mode was achieved by employing a hydrophobic silica dusting agent containing carbon black as an agent to act as an enhancing matrix. The use of the hydrophobic silica dusting agent results in developed marks used for locating/visualising the prints and also acts as a SALDI-TOF-MS enhancer which is equivalent to the standard matrix enhancer 2,5-dihydroxybenzoic acid. This technique has been employed for the analysis of latent fingermarks for contact residues on fingers, and for detection of illicit drugs for both parent drugs and their metabolites. Direct MS analysis was performed on the pre-dusted fingermarks on the surface of a target plate. In addition, after lifting using commercial tape, MS analyses were made on the lifted marks. When 19 commercial powders were employed, only three produced MS spectra and with intensities less than those produced with the new powder. The presence of the parent drug and its metabolites was confirmed using SALDI-TOF-MS-MS following high energy collision induced dissociation. Characteristic and unique fragmentation patterns were noted in each case. Subsequently, the distribution of these drugs on fingermarks was demonstrated using commercially available imaging software

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***Invitro* evaluation of new biocompatible coatings for solid-phase microextraction: Implications for drug analysis and *in vivo* sampling applications**
In order to to address the limitations of the currently available coatings, a new line of solid-phase microextraction (SPME) coatings suitable for use with liquid chromatography applications was recently produced. The developed coatings were immobilized on the metal fiber core and consisted of a mixture of proprietary biocompatible binder and various types of coated silica (octadecyl, polar embedded and cyano) particles. The *in vitro* assessment of these new SPME fibers was investigated in order to evaluate their suitability for drug analysis and *in vivo* SPME applications. The primary parameters examined were extraction efficiency, solvent resistance, preconditioning, dependence of extraction kinetics on coating thickness, carryover, linear range and inter-fiber reproducibility. The performance of the proposed coatings was compared with commercial Carbowax-TPR (CW-TPR) coating, where applicable. The fibers were examined for the extraction of drugs of different classes (carbamazepine, propranolol, pseudoephedrine, ranitidine and diazepam) from plasma and urine. Analyses were carried out emplying liquid chromatography-tandem mass spectrometry. Results suggest that the fibers perform very well for the extraction of biological fluids with no sample pre-treatment required and may also be utilised for *in vivo* sampling applications of flowing blood. A coating thickness of 45 μm was noted to be a suitable compromise between extraction capacity and extraction kinetics. The high extraction efficiency of these coatings, pre-equilibrium SPME with very short extraction times (2 min) indicates that they may be employed to increase sample throughput. Inter-fiber reproducibility was < or = 11% R.S.D. (n=10) for model drugs investigated in plasma. This is a significant improvement over polypyrrole coatings reported in literature and facilitates single fiber use for *in vivo* applications

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Determination of *Aconitum* alkaloids in dietary supplements and raw botanical materials by liquid chromatography/UV detection with confirmation by liquid chromatography/tandem mass spectrometry: Collaborative study
Three *Aconitum* alkaloids (aconitine, mesaconitine, and hypaconitine) were analysed in an interlaboratory study in raw botanical material and dietary supplements. Diethyl ether in the presence of ammonia was employed for extraction. Following cleanup utilising solid-phase extraction to remove matrix interferences, the alkaloids were determined by reversed-phase liquid chromatography (LC)/UV detection at 235 nm with confirmation by LC/tandem mass spectrometry (MS/MS). A total of 14 blind duplicates were successfully analyzed by 12 collaborators. For repeatability, the relative standard deviation (RSDr) values ranged from 1.9 to 16.7%, and for reproducibility, the RSDR values ranged from 6.5 to 33%. The HorRat values were all <2 with only one exception at 2.3. All collaborating laboratories had calibration curves with correlation coefficients of >0.998. Furthermore, 6 collaborators performed the confirmation and were able to verify the identities of the alkaloids by using LC/MS/MS

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Extraction and determination of some psychotropic drugs in urine samples using dispersive liquid-liquid microextraction followed by high-performance liquid chromatography
Analysis of three psychotropic drugs (amitryptiline, clomipramine and thioridazine) in urine samples has been performed with a simple, rapid and sensitive technique termed dispersive liquid-liquid microextraction (DLLME) combined with high-performance liquid chromatography-ultraviolet detector (HPLC-UV). A C_8 column operating under the optimal chromatographic conditions (mobile phase: ammonium acetate (0.03 mol/l, pH 5.5)-acetonitrile (60:40, v/v); flow rate: 1.0 ml/min; detection wavelength: 238 nm) was employed. Parameters such as pH, extraction and disperser solvent type and their volume, extraction time and ion strength were determined and optimized for extraction efficiency. Absolute recoveries of amitryptiline, clomipramine and thioridazine from the urine samples were 96, 97 and 101%, respectively when DLLME conditions were optimised. Detection limits (LODs) and quantification (LOQs) were 3 and 10 ng/ml for amitryptiline, 7 and 21 ng/ml for clomipramine, and 8 and 25 ng/ml for thioridazine, respectively. For nine replicate determinations, the relative standard deviations (RSDs) at 0.100 μg/ml level of target drugs were less than 4.8%. Good linear behaviors over the investigated concentration ranges were produced with the values of $r^2 > 0.998$ for the target drugs. Real urine samples from two female patients under amitryptiline and clomipramine treatment respectively were successfully analysed

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Simultaneous determination of androstenedione, 11 -hydroxyandrostenedione, and testosterone in human plasma by stable isotope dilution mass spectrometry
A GC-MS technique for the simultaneous analysis of androstenedione (AD), 11β-hydroxyandrostenedione (11β-OHAD), and testosterone (TS) in human plasma is described. [19,19,19-$^2H_3$]Androstenedione (AD-$^2H_3$), 11β-hydroxy-[1,2,4,19-$^{13}C_4$]androstenedione (11β-OHAD-$^{13}C_4$), and [1,16,16,17-$^2H_4$]testosterone (TS-$^2H_4$) were employed as internal standards. Pentafluoropropionic (PFP) derivatization with good GC behavior was utilised for the GC-MS analysis of the three steroids. The detection limit of the present GC-MS-SIM method was found to be 1 pg per injection for AD (S/N ratio=4.5), 5 pg for 11β-OHAD (S/N ratio=5.0), and 1 pg for TS (S/N ratio=4.4), respectively. Calibration curves were linear from 0.22 to 2.80 ng/ml (r=0.9998) for AD, from 0.56 to 3.19 ng/ml (r=0.9996) for 11β-OHAD, and from 2.05 to 10.3 ng/ml (r=0.9996) for TS. The intra- and inter-day assay reproducibilities of the three androgens determined were in good agreement with the real amounts added. The relative errors (R.E.) were -3.1 to 2.4%. The inter-assay relative standard deviation (R.S.D.) was less than 5.3%. This technique is sensitive and reliable for the simultaneous determination of AD, 11β-OHAD, and TS in plasma. The method may be of particular use in evaluating the conversion of AD to 11β-OHAD and the interconversion of AD and TS in humans

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