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Current awareness in drug testing and analysis

1 Reviews

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J Mass Spectrom 2008 **43** (7) 903
Special feature: Commentary - New and old challenges of sports drug testing
This brief note gives a general overview on the activity of the antidoping laboratories accredited by the World Anti-Doping Agency (WADA), outlining the evolution, over the last four decades, of the analytical methods and techniques in the detection of prohibited substances and methods. Special emphasis is given to the future trends of the fight against doping in sports, as seen from the perspective of a laboratory scientist, in the wider context of fair play, health protection, and perception of the activity of the antidoping laboratories by the general public

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Mass Spectrom Rev 2008 **27** (4) 378
Mass spectrometric analysis of illicit drugs in wastewater and surface water
Residues of illicit drugs have been recently found in urban wastewater and surface water. Their levels reflect the amount of drugs collectively excreted by consumers and can therefore be used to estimate drug abuse. An overview of the most widely used illicit drugs and of the analytical methods used for their detection in wastewater and surface water is presented here. Solid-phase extraction and high performance liquid chromatography-tandem mass spectrometry are the techniques that have been used for these investigations. Instrumental conditions and fragmentation patterns of illicit drugs and their metabolites are described

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J Mass Spectrom 2008 **43** (7) 854
Special feature: Tutorial - The application of carbon isotope ratio mass spectrometry to doping control
The administration of synthetic steroid copies is one of the most important issues facing sports. Doping control laboratories accredited by the World Anti-Doping Agency (WADA) require methods of analysis that allow endogenous steroids to be distinguished from their synthetic analogs in urine. The ability to measure isotope distribution at natural abundance with high accuracy and precision has increased the application of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) to doping control in recent years. GC-C-IRMS is capable of measuring the carbon isotope ratio ($\delta^{13}C$) of urinary steroids and confirm their synthetic origin based on the abnormal ^{13}C content. This tutorial describes some of the complexities encountered by obtaining valid $\delta^{13}C$ measurements from GC-C-IRMS and the need for careful interpretation of all relevant information concerning an individual's metabolism in order to make an informed decision with respect to a doping violation

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J Mass Spectrom 2008 **43** (11) 1441
Special feature: Perspective - Mass spectrometry in biodefense
Potential agents for biological attacks include both microorganisms and toxins. In mass spectrometry (MS), rapid identification of potential bioagents is achieved by detecting the masses of unique biomarkers, correlated to each agent. Currently, proteins are the most reliable biomarkers for detection and characterization of both microorganisms and toxins, and MS-based proteomics is particularly well suited for biodefense applications. Confident identification of an organism can be achieved by top-down proteomics following identification of individual protein biomarkers from their tandem mass spectra. In bottom-up proteomics, rapid digestion of intact protein biomarkers is again followed by MS/MS to provide unambiguous bioagent identification and characterization. Bioinformatics obviates the need for culturing and rigorous control of experimental variables to create and use MS fingerprint libraries for various classes of bioweapons. For specific applications, MS methods, instruments and algorithms have also been developed for identification based on biomarkers other than proteins and peptides

Geyer H, Parr MK, Koehler K, Mareck U, Schanzer W, Thevis M// German Sport Univ Cologne, Ctr Prevent Doping Res, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany
J Mass Spectrom 2008 **43** (7) 892
Nutritional supplements cross-contaminated and faked with doping substances
Since 1999 several groups have analyzed nutritional supplements with mass spectrometric methods (GC/MS, LC/MS/MS) for contaminations and adulterations with doping substances. These investigations showed that nutritional supplements contained prohibited stimulants as ephedrines, caffeine, methylenedioxymetamphetamine and sibutramine, which were not declared on the labels. An international study performed in 2001 and 2002 on 634 nutritional supplements that were purchased in 13 different countries showed that about 15% of the nonhormonal nutritional supplements were contaminated with anabolic-androgenic steroids (mainly prohormones). Since 2002, also products intentionally faked with high amounts of 'classic' anabolic steroids such as metandienone, stanozolol, boldenone, dehydrochloromethyl-testosterone, oxandrolone etc. have been detected on the nutritional supplement market. These anabolic steroids were not declared on the labels either. The sources of these anabolic steroids are probably Chinese pharmaceutical companies, which

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.

sell bulk material of anabolic steroids. In 2005 vitamin C, multivitamin and magnesium tablets were confiscated, which contained cross-contaminations of stanozolol and metandienone. Since 2002 new 'designer' steroids such as prostanozol, methasterone, androstatrienedione etc. have been offered on the nutritional supplement market. In the near future also cross-contaminations with these steroids are expected. Recently a nutritional supplement for weight loss was found to contain the β2-agonist clenbuterol. The application of such nutritional supplements is connected with a high risk of inadvertent doping cases and a health risk. For the detection of new 'designer' steroids in nutritional supplements, mass spectrometric strategies (GC/MS, LC/MS/MS) are presented

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J Mass Spectrom 2008 **43** (7) 839
Special feature: Historical - History of mass spectrometry at the Olympic Games
Mass spectrometry has played a decisive role in doping analysis and doping control in human sport for almost 40 years. The standard of qualitative and quantitative determinations in body fluids has always attracted maximum attention from scientists. With its unique sensitivity and selectivity properties, mass spectrometry provides state-of-the-art technology in analytical chemistry. Both anti-doping organizations and the athletes concerned expect the utmost endeavours to prevent false-positive and false-negative results of the analytical evidence. The Olympic Games play an important role in international sport today and are milestones for technical development in doping analysis. This review of the part played by mass spectrometry in doping control from Munich 1972 to Beijing 2008 Olympics gives an overview of how doping analysis has developed and where we are today. In recognizing the achievements made towards effective doping control, it is of the utmost importance to applaud the joint endeavours of the World Anti-Doping Agency, the International Olympic Committee, the international federations and national anti-doping agencies to combat doping. Advances against the misuse of prohibited substances and methods, which are performance-enhancing, dangerous to health and violate the spirit of sport, can be achieved only if all the stakeholders work together

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J Mass Spectrom 2008 **43** (7) 877
Factors influencing the steroid profile in doping control analysis
Steroid profiling is one of the most versatile and informative screening tools for the detection of steroid abuse in sports drug testing. Concentrations and ratios of various endogenously produced steroidal hormones, their precursors and metabolites including testosterone (T), epitestosterone (E), dihydrotestosterone (DHT), androsterone (And), etiocholanolone (Etio), dehydroepiandrosterone (DHEA), 5α-androstane-3α,17β-diol (Adiol), and 5β-androstane-3α,17β-diol (Bdiol) as well as androstenedione, 6α-OH-androstenedione, 5β-androstane-3α,17α-diol (17-epi-Bdiol), 5α-androstane-3α,17α-diol (17-epi-Adiol), 3α,5-cyclo-5α-androstan-6β-ol-17-one (3α,5-cyclo), 5α-androstanedione (Adion), and 5β-androstanedione (Bdion) add up to a steroid profile that is highly sensitive to applications of endogenous as well as synthetic anabolic steroids, masking agents, and bacterial activity. Hence, the knowledge of factors that do influence the steroid profile pattern is a central aspect, and pharmaceutical (application of endogenous steroids and various pharmaceutical preparations), technical (hydrolysis, derivatization, matrix), and biological (bacterial activities, enzyme side activities) issues are reviewed

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Anal Chem 2008 **80** (12) 4373
Environmental mass spectrometry: Emerging contaminants and current issues
No abstract available

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J Mass Spectrom 2008 **43** (7) 865
Mass spectrometry of selective androgen receptor modulators
Nonsteroidal selective androgen receptor modulators (SARMs) are an emerging class of drugs for treatment of various diseases including osteoporosis and muscle wasting as well as the correction of age-related functional decline such as muscle strength and power. Several SARMs, which have advanced to preclinical and clinical trials, are composed of diverse chemical structures including arylpropionamide-, bicyclic hydantoin-, quinoline-, and tetrahydroquinoline-derived nuclei. Since January 2008, SARMs have been categorized as anabolic agents and prohibited by the World Anti-Doping Agency (WADA). Suitable detection methods for these low-molecular weight drugs were based on mass spectrometric approaches, which necessitated the elucidation of dissociation pathways in order to characterize and identify the target analytes in doping control samples as well as potential metabolic products and synthetic analogs. Fragmentation patterns of representatives of each category of SARMs after electrospray ionization (ESI) and collision-induced dissociation (CID) as well as electron ionization (EI) are summarized. The complexity and structural heterogeneity of these drugs is a daunting challenge for detection methods

2 Sports Doping - General

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Biomed Chromatogr 2008 **22** (7) 695
Rapid screening of polysaccharide-based plasma volume expanders dextran and hydroxyethyl starch in human urine by liquid chromatography-tandem mass spectrometry
The increasing number of samples and target substances in doping control requires continuously improved screening methods, combining high-throughput analysis, simplified sample preparation, robustness and reliability. Hence, a rapid screening procedure based on liquid chromatography-electrospray ionization-tandem mass spectrometry with in-source collision-induced dissociation was developed. The detection of the polysaccharide-based plasma volume expanders dextran and hydroxyethyl starch (HES) in human urine was established without further sample preparation. The in-source fragmentation strategy of the approach represented a valuable tool in the analysis of the polysaccharide-based compounds, allowing the use of tandem mass spectrometry. After direct injection of urine specimens, analytes were chromatographically separated on a monolithic reverse-phase column and detected via multiple reaction monitoring of diagnostic ions at detection limits of 10 μg/ml for HES and 30 μg/ml for dextran. Validation was performed regarding the parameters specificity, linearity, precision (8-18%) and accuracy (77-105%) and the method was applied to the investigation of approximately 400 doping control samples and seven dextran and two hydroxyethyl starch post-administration samples. The approach demonstrated its capability as a rapid screening tool for the detection of dextran and hydroxyethyl starch and represents an alternative to existing screening procedures since time consuming hydrolysis or derivatization steps were omitted

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Anal Bioanal Chem 2008 **392** (4) 681
A screening method for the simultaneous detection of glucocorticoids, diuretics, stimulants, anti-oestrogens, beta-adrenergic drugs and anabolic steroids in human urine by LC-ESI-MS/MS
A general screening prodeure based on liquid chromatography/mass spectrometry (LC/MS), is described for the simultaneous detection in human urine of 72 xenobiotics (21 diuretics, 16 synthetic glucocorticoids, 17 beta-adrenergic drugs, 10 stimulants, 5 anti-oestrogens and 3 anabolic steroids), excreted free or as glucuro-conjugates in urine. The method has been specifically designed and evaluated in view of its potential application to anti-doping analyses but it may also be effective in other areas of analytical toxicology. Sample preparation was based on two liquid/liquid separation steps (performed at alkaline and at acid pH, respectively) of hydrolyzed human urine, and then an assay by LC/MS-MS in positive and negative ionization mode using an electrospray ionization source (ESI) and multiple reaction monitoring (MRM) as the acquisition mode. The overall time needed for an LC run was less than 15 minutes. All compounds exhibited good reproducibility in terms of both the retention times (CV%<1) and the relative abundances of the diagnostic transitions (CV%<10). The limits of detection (LOD) were in the range of 1-50 ng/ml for glucocorticoids, anti-oestrogens and steroids, and 50-500 ng/ml for diuretics, beta-adrenergic drugs and stimulants, thereby meeting the minimum required performance limits (MRPL) set by the World Anti-Doping Agency (WADA) for the accredited anti-doping laboratories

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J Mass Spectrom 2008 **43** (7) 949
Introduction of HPLC/orbitrap mass spectrometry as screening method for doping control
A new doping control screening method has been developed, for the analysis of doping agents in human urine, using HPLC/orbitrap with in-source collision-induced dissociation and atmospheric pressure chemical ionization. The developed method allows the detection of 29 compounds, including agents with antiestrogenic activity, β_2 agonists, exogenous anabolic steroids, and other anabolic agents. The mass accuracy of this method is better at 2 ppm using an external reference. The detection limit for all compounds tested was better than 100 pg/ml. The recoveries of most analytes were above 70%. The measured median repeatability values for doping agents included in the method at concentrations of 1 and 10 ng/ml were 21 and 17%, respectively. The relative standard deviation (RSD) of the intraday precision (n = 6) ranged from RSD = 16-22%, whereas the interday precision (n = 18), ranged from RSD = 17-26%, depending on the solute concentration investigated

3 Steroids

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J Mass Spectrom 2008 **43** (7) 993

Quantitative confirmation of testosterone and epitestosterone in human urine by LC/Q-ToF mass spectrometry for doping control

Testosterone (T) is the primary male sex hormone. In addition to the development of secondary sex characteristics, testosterone has anabolic effects including increases in muscle size and strength and increases in lean body mass, making it an attractive candidate to enhance athletic performance. In the case of exogenous administration of testosterone, the ratio of testosterone to its isomer, epitestosterone (E), is elevated. WADA has set a standard for T/E ratios of 4.0 as indicative of possible exogenous testosterone administration. Typically, a sample that screens for a T/E ratio above that threshold is then subjected to quantitative confirmation by GC/MS. This methodology, however, can limited due to sensitivity issues as well as a limited number of qualifying ions that can be used for unambiguous identification. We have developed a confirmation method which uses liquid/liquid extraction, followed by room temperature Girard P derivatization, and analysis using LC/MS-ToF. We observe a number of advantages over conventional GC/MS analysis. Analysis time is decreased. Sensitivity is increased, resulting in limits of detection of 2 and 0.5 ng/ml for testosterone and epitestosterone, respectively. The number of diagnostic qualifier ions is also increased allowing more confident identification of the analytes. Finally, while this method has been developed on a QToF instrument, it should be easily transferable to any tandem LC/MS/MS system

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J Mass Spectrom 2008 **43** (7) 958

Synthesis and identification of hydroxylated metabolites of the anti-estrogenic agent cyclofenil

The detection of metabolites of the anti-estrogenic substance cyclofenil, listed on the World Anti-Doping Agency (WADA) Prohibited List since 2004 is described. Target substances are hydroxylated metabolites, bearing an aliphatic hydroxyl group either in the 2-, 3- or 4-position of the aliphatic ring, in addition to the phenolic functions on the aromatic rings. Structural identification used NMR as well as high-resolution mass spectrometry after nano-electrospray ionisation (ESI). Unambiguous detection of all three synthesised cyclofenil metabolites M1-M3 was done using gas chromatography for separation and electron ionisation mass spectrometry for detection of the per-silylated compounds in comparison with a reference urine deriving from an excretion study within the World Anti-Doping Agency 2007 Educational Programme

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J Mass Spectrom 2008 **43** (7) 965

Liquid chromatographic-mass spectrometric analysis of glucuronide-conjugated anabolic steroid metabolites: Method validation and inter-laboratory comparison

Liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method for simultaneous and direct detection of 12 glucuronide-conjugated anabolic androgenic steroid (AAS) metabolites in human urine is described. The compounds selected were the main metabolites detected in human urine after dosing of the most widely abused AAS in sports, e.g. methandienone, methenolone, methyltestosterone, nandrolone and testosterone, and certain deuterium-labeled analogs of these metabolites. Sample preparation and the LC-ESI-MS/MS method were optimized, validated, and the overall process was implemented and the results between seven laboratories were compared. All the metabolites were extracted simultaneously by solid-phase extraction (SPE) and analyzed by LC-ESI-MS/MS with positive ionization mode and multiple reaction monitoring (MRM). Recovery of the SPE for the AAS glucuronides was 89-100% and ten out of twelve compounds had detection limits in the range of 1-10 ng/ml in urine. The results for inter/intraday repeatability were satisfactory and the interlaboratory comparison with authentic urine samples demonstrated the ease of method transfer from one instrument setup to another. When equivalent triple quadrupole analyzers were employed the overall performance was independent from instrument manufacturer, electrospray ionisation (ESI) or atmospheric pressure chemical ionization (APCI) and liquid chromatographic (LC) column, whereas major differences were encountered when changing from one analyzer type to another, especially in the analysis of those AAS glucuronides ionized mainly as adducts

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Rapid Commun Mass Spectrom 2008 **22** (14) 2161

Determination of $^{13}C/^{12}C$ ratios of endogenous urinary steroids: Method validation, reference population and application to doping control purposes

The application of a comprehensive gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS)-based method for stable carbon isotopes of endogenous urinary steroids is presented. The key element in sample preparation is the consecutive cleanup with high-performance liquid chromatography (HPLC) of underivatized and acetylated steroids, which allows the isolation of ten analytes (11β-hydroxyandrosterone, 5α-androst-16-en-3β-ol, pregnanediol, androsterone, etiocholanolone, testosterone, epitestosterone, 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol and dehydroepiandrosterone) from a single urine specimen. These steroids are of particular importance to doping controls as they enable the sensitive and retrospective detection of steroid abuse by athletes.Depending on the biological background, the determination limit for all steroids ranges from 5 to 10 ng/ml for a 10 ml specimen. The method is validated by means of linear mixing models for each steroid, which covers repeatability and reproducibility. Specificity was further demonstrated by gas chromatography/mass spectrometry (GC/MS) for each analyte, and no influence of the sample preparation or the quantity of analyte on carbon isotope ratios was observed. In order to determine naturally occurring $^{13}C/^{12}C$ ratios of all implemented steroids, a reference population of $n = 61$ subjects was measured to enable the calculation of reference limits for all relevant steroidal Delta values

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Anal Chem 2008 **80** (5) 1709

Efficient approach for the comprehensive detection of unknown anabolic steroids and metabolites in human urine by liquid chromatography-electrospray-tandem mass spectrometry

A challenge in doping analysis is the detection of new anabolic steroid metabolites and new designer steroids in urine. An approach to the detection of unknown anabolic steroids and metabolites based on precursor ion scanning for is proposed. Different fragmentation pathways of selected anabolic steroids in MS/MS spectra were revealed at low and medium collision energy depending on the steroid structure. Analysis at high collision energy revealed only three common ions at *m/z* 105, *m/z* 91, and *m/z* 77 for all studied anabolic steroids. These ions can be explained by the fragmentation of the steroid structure and corresponded to the methyl tropylium, tropylium, and phenyl ions, respectively. The theoretical low specificity of these ions and the simultaneous presence of all of them was used as a starting point to consider a substance as a possible anabolic steroid. Therefore, the developed approach was based on the simultaneous acquisition of the precursor ion scan of *m/z* 105, 91, and 77. The specificity of this approach has been confirmed by the injection of several doping agents including β-agonists, corticosteroids, β-blockers, and diuretics. Overall, only compounds with a steroidal structure showed a signal at all three selected *m/z* values although some exceptions have been found. The applicability of the method was tested for three different situations: the detection of steroid metabolites, the detection of unknown steroids, and the analysis of prohormones. In metabolic studies, several recently reported fluoxymesterone metabolites were also found using this method. To detect unknown steroids, some negative urine samples were spiked with the designer steroid THG and 33 other anabolic steroids and treated as blind samples. Finally, the applicability of the developed approach for the analysis of dietary supplements was validated by the analysis of a prohormone where several impurities and/or degradation products were found

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Food Addit Contam 2008 **25** (5) 557

Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection for the determination of anabolic steroids and related compounds in nutritional supplements

The determination of anabolic steroids and related compounds in nutritional supplements has been achieved with a simple and effective analytical method. Target compounds were isolated with ethyl acetate, crude extract was purified using dispersive solid-phase extraction (SPE) with primary secondary amine (PSA) as sorbent, and finally they were identified and quantified as underivatized compounds using two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOF MS). This method was confirmed for 25 steroids in two types of commercially available solid nutritional supplements: protein concentrate and creatine monohydrate. Repeatability expressed as the relative standard deviation of analyte concentration ranged from 4.1 to 20.5%. Recoveries between 70.0 and 122.6% were obtained for the target compounds except for oxymetholone in protein concentrate

where the recovery was low as a result of strong interactions with PSA. Excellent linearity was obtained for six-point calibration with regression coefficients of 0.997-1.000 for all compounds. The limits of quantification ranged from 0.007 to 0.114 mg/kg. For a monitoring programme of 48 samples of nutritional supplements, three were positive. Nandrolone, testosterone, dehydroepiandrosterone (DHEA), 5alpha-androstan-3,17-dione, 19-norandrostendione and progesterone were found in positive samples at concentrations between 0.022 and 0.398 mg/kg

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J Chromatogr A 2008 **1196-1197** 153

Isolation and quantification by high-performance liquid chromatography-ion-trap mass spectrometry of androgen sulfoconjugates in human urine

In addition to steroid glucuronides, sulfoconjugates may be used as markers of steroid administration as well as endogenous steroid production. The simultaneous separation, determination and quantification of sulfate and glucuronide derivatives of testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio) and dehydroepiandrosterone (DHEA) in human urine has been analyzed by a fast and sensitive analytical procedure. First, a weak anion-exchange solid-phase extraction support (SPE Oasis WAX) was used for complete and rapid separation of sulfates and glucuronides in two extracts after loading of urine sample (2 ml). Subsequently, sulfates were analyzed directly by high-performance liquid chromatography-ion-trap mass spectrometry (LC-MS/MS) with electrospray ionization in negative mode. Chromatographic separation of the targeted sulfoconjugates was achieved using a Waters XBridge C_{18} column (150 mm x 4.6 mm I.D., 5 µm) with gradient elution. Assay validation showed good performance for instance for T sulfate (TS) and E sulfate (ES) in terms of trueness (89-107%), repeatability (3.4-22%) and intermediate precision (5.8-22%) over the range of 2-200 ng/ml (corresponding to 1.5-147 ng/ml as free steroids). Results obtained with biological samples demonstrated the validity of this analytical strategy for direct measurement of androgen sulfoconjugates and glucuroconjugates in human urine

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J Mass Spectrom 2008 **43** (5) 639

Mass spectrometry of hydantoin-derived selective androgen receptor modulators

N-Aryl-hydroxybicyclohydantoins represent a new class of tissue-selective anabolic agents [selective androgen receptor modulators (SARMs)] and are promising therapeutics as well as drugs prohibited in amateur and professional sport. The dissociation behavior after negative and positive electrospray ionization (ESI) and subsequent collision-induced dissociation (CID) was studied with a drug candidate (BMS 564929) as well as structurally related and isotope-labeled analogs using high resolution/high accuracy orbitrap mass spectrometry. Positive ionization and CID yielded characteristic product ions resulting from the cleavage of the hydantoin structure providing information about the proline-derived nucleus as well as the substituted aryl residue at *m/z* 96 and 193, respectively. Negative ESI and CID (MS/MS) yielded product ions mainly representing losses of water and CO_2, the latter of which is of particular significance as the hydantoin structure does not contain a carboxyl function. Employing MS^n experiments with accurate mass determination on six model SARMs, dissociation pathways to characteristic product ions were proposed supporting the identification of these drugs, their metabolites or related compounds in future doping control assays

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Rapid Commun Mass Spectrom 2008 **22** (16) 2471

Doping control analysis of tricyclic tetrahydroquinoline-derived selective androgen receptor modulators using liquid chromatography/electrospray ionization tandem mass spectrometry

Selective androgen receptor modulators represent an emerging class of therapeutics to counteract various diseases such as osteoporosis and muscle wasting. Numerous drug candidates have been developed and investigated including a group that comprises a tricyclic tetrahydroquinoline nucleus such as 2-methyl-2-(8-nitro-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[c]chinolin-4-yl)propan-1-ol. Due to their novelty and medicinal purpose, these compounds also possess great potential for misuse in sports, and studies on the mass spectrometric behavior of three synthesized model substances and drug candidates were conducted to provide information on typical dissociation pathways following electrospray ionization and collision-induced dissociation. Product ion mass spectra derived from protonated molecules were studied using high resolution/high accuracy orbitrap mass spectrometry, and characteristic fragmentation routes and product ions were elucidated. Major and general findings include the elimination of a hydroxyl radical from $[M+H]^+$, the elimination of the 2-substituted side chain, and the gas-phase rearrangement of the investigated tricyclic tetrahydroquinolines to 6-nitroquinoline yielding a common product ion at *m/z* 175. Knowledge of these dissociation pathways supports the identification of related substances as well as metabolic products, which is of utmost importance to drug testing laboratories. The compounds were implemented into existing screening procedures, and detection limits (0.2-0.6 ng/ml), recoveries (92-97%), and intraday and interday precision (<22%) were evaluated

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Steroids 2008 **73** (7) 751

Gas chromatography-mass spectrometry method for the analysis of 19-nor-4-androstenediol and metabolites in human plasma: Application to pharmacokinetic studies after oral administration of a prohormone supplement

The WADA list of prohibited classes of substances includes nandrolone and its prohormone 19-nor-4-androstenediol. The aim of this study is to investigate the plasma levels of 19-nor-4-androstenediol and its metabolites after oral administration of a nutritional supplement containing the drug. Two capsules of Norandrodiol Select 300 were orally administered to six healthy male volunteers. Plasma samples were collected up to 24h. Samples were extracted to obtain free and glucuronoconjugated metabolic fractions. Trimethylsilyl derivatives of both fractions were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The method was validated to determine linearity, extraction recovery, limit of detection and quantification, intra- and inter-day precision and accuracy. After administration of 19-nor-4-androstenediol, the main metabolites detected were norandrosterone and noretiocholanolone, primarily in the glucuronide fraction. Nandrolone, norandrostenedione and 19-nor-4-androstenediol were also detected at lower concentrations

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Rapid Commun Mass Spectrom 2008 **22** (19) 3035

Urinary metabolic profile of 19-norsteroids in humans: Glucuronide and sulphate conjugates after oral administration of 19-nor-4-androstenediol

19-Nor-4-androstenediol (NOL) is a prohormone of nandrolone (ND). Both substances are included in the WADA List of Prohibited Classes of Substances and their administration is determined by the presence of 19-norandrosterone (NA) with the urinary threshold concentration of 2 ng/ml. Routine analytical procedures allow the determination of NA excreted free and conjugated with glucuronic acid, but amounts of ND and NOL metabolites are also excreted in the sulphate fraction. The aim of this study is to determine the urinary metabolic profile after oral administration of a nutritional supplement containing NOL. Urine samples were collected up to 96 h following supplement administration and were extracted to obtain separately three metabolic fractions: free, glucuronide and sulphate. Extraction with tert-butyl methyl ether was performed after the hydrolysis steps and trimethylsilyl derivatives were analyzed by gas chromatography/mass spectrometry (GC/MS). After oral administration of NOL, the main metabolites detected were NA and noretiocholanolone (NE) in the glucuronide and sulphate fractions. The relative abundances of each metabolite in each fraction fluctuate with time; a few hours after administration the main metabolite was NA glucuronide whereas in the last sample (4 days after administration) the main metabolite was the NA sulphate and the second was the NE glucuronide. During the studied period almost half of the dose was excreted and the main metabolites were still found in urine after 96 h. Norepiandrosterone and norepietiocholanolone were also detected only in the sulphate fraction. Our results suggest that sulphate metabolites should be taken into consideration in order to increase the retrospectivity in the detection of 19-norsteroids after oral administration

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Steroids 2008 **73** (11) 1143

Detection of anabolic steroids abuse using a yeast transactivation system

Gas chromatography followed by mass spectrometry (GC/MS) is the classical analytical method for detection of anabolic steroid abuse. However, even molecules with a chemical structure typical for this class of substances, are sometimes not identifiable in routine screening by GC/MS when their precise chemical structure is still unknown. Identification of anabolic steroids based on their biological activity could provide a supplementary approach to identify anabolic steroid abuse and could be a structure-independent. To develop of such a system, we have analyzed the yeast androgen receptor (AR) reporter gene system to identify anabolic steroids in human urine samples. The yeast reporter gene system was able to detect a variety of different anabolic steroids dissolved in buffer and their metabolites with high specificity, including the so-called 'designer steroid' tetrahydrogestrinone. By contrast, other non-androgenic steroids, like glucocordicoids, progestins, mineralocordicoids and estrogens had a low potency to stimulate transactivation. To examine whether the system would also allow the detection of androgens in urine, experiments with spiked urine samples were performed. The androgen reporter gene in yeast responds very sensitive to 5α-dihydrotestosterone (DHT), even at high urine concentrations.

To investigate whether the test system would also be able to detect anabolic steroids in the urine of anabolic steroid abusers, anonymous urine samples previously characterized by GCMS were analyzed with the reporter gene assay. Even when the concentration of the anabolic metabolites was comparatively low in some positive samples it was possible to identify the majority of positive samples by their biological activity. In conclusion, the results demonstrate that the yeast reporter gene system detects anabolic steroids and corresponding metabolites with high sensitivity even in urine of anabolic steroid abusing athletes. Therefore, this system may be developed towards a powerful (pre) screening tool for the established doping tests. The technique is easy to handle, robust, cost-efficient and needs no high-tech equipment. But most importantly, a biological test system does not require knowledge of the chemical structure of androgenic substances. Consequently,it is suitable to detect previously unidentified substances, especially those of the class of so-called designer steroids

4 Peptides

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J Mass Spectrom 2008 **43** (7) 924

Low LC-MS/MS detection of glycopeptides released from pmol levels of recombinant erythropoietin using nanoflow HPLC-chip electrospray ionization

The test used by anti-doping laboratories to detect the misuse of recombinant erythropoietin (rhEPO) is based on its different migration pattern on isoelectric focusing (IEF) gel compared with the endogenous human erythropoietin (hEPO) that can possibly be explained by structural differences. While there is definitely a need to identify those differences by LC-MS/MS, the extensive characterization that was achieved for the rhEPO was never performed on human endogenous EPO because its standard is not available in sufficient amount. The goal of this study was to develop an analytical method to detect pmol amounts of *N*-linked and *O*-linked glycopeptides of the recombinant hormone as a model. Using a nanoflow HPLC-Chip electrospray ionization/ion trap mass spectrometer, the diagnostic ion at *m/z* 366 of oligosaccharides was monitored in the product ion spectra to identify the four theoretical glycosylation sites, Asn24, Asn38, Asn83 and Ser126, respectively, on glycopeptides 22-37, 38-55, 73-96 and 118-136. With 3 pmol of starting material applied on Chip, only the desialylated *N*-glycopeptides 22-37 and 38-55/38-43 could be observed, and of all the glycan isoforms, those with the smaller structures were predominantly detected. While the preservation of the sialic acid moieties decreased the detection of all the *N*-glycopeptides, it allowed a more extensive characterization of the *O*-linked glycopeptide 118-136. The technique described herein provides a mean to detect glycopeptides from commercially available pharmaceutical preparations of rhEPO with the sensitivity required to analyze pmol amounts of hEPO, which could ultimately lead to the identification of structural differences between the recombinant and the human forms of the hormone

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Rapid Commun Mass Spectrom 2008 **22** (20) 3255

Enrichment of low molecular weight serum proteins using acetonitrile precipitation for mass spectrometry based proteomic analysis

A rapid acetonitrile (ACN)-based extraction method has been developed that reproducibly depletes high abundance and high molecular weight proteins from serum prior to mass spectrometric analysis. A nanoflow liquid chromatography/tandem mass spectrometry (nano-LC/MS/MS) multiple reaction monitoring (MRM) method for 57 high to medium abundance serum proteins was used to characterise the ACN-depleted fraction after tryptic digestion. Of the 57 targeted proteins 29 were detected and albumin, the most abundant protein in serum and plasma, was identified as the 20th most abundant protein in the extract. The combination of ACN depletion and one-dimensional nano-LC/MS/MS enabled the detection of the low abundance serum protein, insulin-like growth factor-I (IGF-I), which has a serum concentration in the region of 100 ng/ml. One-dimensional sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the depleted serum showed no bands corresponding to proteins of molecular mass over 75 kDa after extraction, demonstrating the efficiency of the method for the depletion of high molecular weight proteins. Total protein analysis of the ACN extracts showed that approximately 99.6% of all protein is removed from the serum. The ACN-depletion strategy offers a viable alternative to the immunochemistry-based protein-depletion techniques commonly used for removing high abundance proteins from serum prior to MS-based proteomic analyses

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J Mass Spectrom 2008 **43** (7) 936

Can glycans unveil the origin of glycoprotein hormones? - Human chorionic gonadotrophin as an example -

Doping with (glyco)protein hormones represent an extremely challenging, analytical problem as nearly all are constitutively present at low concentrations that fluctuate according to circadian or alternative periodical, or external stimuli. Thus the mere concentration in a biological sample is only resolutive when this surpasses extreme values. As the vast majority of these molecules are produced by recombinant DNA technology it is believed that the exogenous molecules could bear the signature of the host cell. In particular, these could comprise structural differences originated from co or post-translational differences. In this study we have employed both proteomics and glycomics strategies to compare recombinant and urinary human chorionic gonadotrophin in order to evaluate this hypothesis. As anticipated the recombinant hormone could be shown to contain *N*-glycolyl neuraminic acid, a sialic acid that cannot be produced by humans. Furthermore, differences were observed in the overall glycosylation, in particular the presence of abundant hybrid-type glycans that were much less pronounced in the recombinant species. These differences were determined to occur predominantly in the α-subunit for which antidoping strategies focussed on these elements could be used for both chorionic gonadotrophin and lutrophin as they share the same α-subunit

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J Mass Spectrom 2008 **43** (7) 916

Identification of zinc- -2-glycoprotein binding to clone AE7A5 antihuman EPO antibody by means of nano-HPLC and high-resolution high-mass accuracy ESI-MS/MS

The detection of doping with recombinant erythropoietins (Epo) by isoelectric focusing (IEF) and Western double blotting strongly relies on the specificity of the detection antibody used. Currently a monoclonal mouse antibody (clone AE7A5) is used for that purpose. Despite its excellent sensitivity (amol range) the antibody shows some nonspecific binding behavior. However, the binding occurs outside the currently used pH range for evaluating erythropoietin IEF profiles. A shotgun proteomics approach is described consisting of preparative IEF on large-sized carrier ampholyte gels (pH 3-5), SDS-PAGE, Western single and double blotting, on-membrane elution of intact proteins, on-membrane and in-solution tryptic digestions, as well as nano-HPLC peptide separation and high-resolution high-mass accuracy ESI-MS/MS peptide sequencing. The nonspecifically interacting protein could be identified as zinc-α-2-glycoprotein (ZAG). Confirmation analyses were performed using recombinant ZAG (rhZAG) and a monoclonal anti-ZAG antibody. It could be demonstrated that the binding of the monoclonal antihuman EPO antibody (clone AE7A5) to ZAG occurs in a highly concentration-dependant manner and that only samples containing increased amounts of urinary ZAG lead to a detectable interaction of the AE7A5 antibody on Epo-IEF gels

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J Mass Spectrom 2008 **43** (7) 908

Mass spectrometric determination of gonadotrophin-releasing hormone (GnRH) in human urine for doping control purposes by means of LC-ESI-MS/MS

The decapeptide gonadotrophin-releasing hormone (GnRH) is endogenously produced in the hypothalamus and secreted into the microcirculation between hypothalamus and pituitary gland. Here, the bioactive hormone is responsible for the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the systemic circulation. Because an intermittent application of exogenous GnRH in young males increases the testosterone plasma level by stimulation of the Leydig cells, the potential misuse of the administered substance offers a reasonable relevancy for doping controls and is prohibited in accordance to the list of banned substances of the World Anti-Doping Agency (WADA). The presented method provides a mass spectrometric approach to determine the nondegraded hormone in regular doping control samples by utilizing a sample preparation procedure with solid phase extraction, immunoaffinity purification and a subsequent separation by liquid chromatography with ESI-MS/MS detection. For liquid chromatography/mass spectrometry two alternative instrumental equipments were tested: the first consisted of an Agilent 1100 liquid chromatograph coupled to an Applied Biosystem Q Trap 4000 mass spectrometer, the second equipment was assembled by a Waters Aquity nano-UPLC coupled to a Thermo LTQ Orbitrap high resolution/high accuracy mass spectrometer. In urine specimens provided from healthy volunteers GnRH was not detected in accordance to the recent literature, but in postadministration samples urinary concentrations between 20 to 100 pg/ml of the intact peptide were determined. The method offered good validation results considering the parameter specificity, linearity (5-300 pg/ml), limit of detection (LOD, approx. 5 pg/ml), precision (inter/intraday, <20%) and accuracy (105%) using Des-pGlu(1)-GnRH as internal standard to control each sample preparation step

5 Diuretics

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J Mass Spectrom 2008 **43** (7) 980
A high-throughput multicomponent screening method for diuretics, masking agents, central nervous system (CNS) stimulants and opiates in human urine by UPLC-MS/MS
A simple and rapid multicomponent screening method of 130 substances for direct injections of urine samples has been developed. The fully automated method based on ultra-performance liquid chromatography (UPLC) and tandem mass spectrometry (MS/MS) is used for three different classes of doping agents: diuretics, central nervous system stimulants (CNS stimulants) and opiates. The samples are diluted with buffer containing internal standards (IS) by a pipetting robot system into 96-well plates. Samples are injected on a reversed phase sub 2-µm particle column connected to a fast polarity switching and rapid scanning tandem mass spectrometer with an electrospray interface. The software used to evaluate the results produced reports containing a small-sized window for each component and a data table list with flags to indicate any adverse analytical findings in the sample. The report can also be processed automatically using an application software, which interpret the data and indicate if there is a suspicious sample. One 96-well plate can be analyzed within 16 h

7 Equine

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J Mass Spectrom 2008 **43** (7) 1001
Hair analysis of anabolic steroids in connection with doping control - Results from horse samples
Doping control of anabolic substances is normally carried out with urine samples taken from athletes and horses. Investigation of alternative specimens, e.g. hair samples, is restricted to special cases, but can also be worthwhile, in addition to urine analysis. Moreover, hair material is preferred in cases of limited availability or complicated collection of urine samples, e.g. from horses. In this work, possible ways of interpretation of analytical results in hair samples are discussed and illustrated by practical experiences. The results demonstrate the applicability of hair analysis to detect anabolic steroids and also to obtain further information about previous abuse. Moreover, the process of incorporation of steroids into hairs is described and the consequences on interpretation are discussed, e.g. on the retrospective estimation of the application date. The chosen examples deal with the detection of the anabolic agent testosterone propionate. Hair samples of an application study, as well as a control sample taken from a racing horse, were referred to. Hair material was investigated by a screening procedure including testosterone, nandrolone and several esters (testosterone propionate, phenylpropionate, decanoate, undecanoate, cypionate; nandrolone decanoate, dodecanoate and phenylpropionate; limits of detection (LODs) between 0.1 and 5.0 pg/mg). Confirmation of testosterone propionate (LOD 0.1 pg/mg) was carried out by an optimised sample preparation. Trimethylsilyl (TMS) and tert-butyl dimethylsilyl derivatives were detected by gas chromatography-high-resolution mass spectrometry (GC-HRMS) and gas chromatography-tandem mass spectrometry (GC-MS/MS)

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Rapid Commun Mass Spectrom 2008 **22** (19) 2999
Quantification of 19-nortestosterone sulphate and boldenone sulphate in urine from male horses using liquid chromatography/tandem mass spectrometry
Following administration of the anabolic steroid 19-nortestosterone or its esters to the horse, a major urinary metabolite is 19-nortestosterone-17β-sulphate. The detection of 19-nortestosterone in urine from untreated animals has led to it being considered a naturally occurring steroid in the male horse. Recently, we have demonstrated that the majority of the 19-nortestosterone found in extracts of 'normal' urine from male horses arises as an artefact through decarboxylation of the 19-carboxylic acid of testosterone. The aim of this investigation was to establish if direct analysis of 19-nortestosterone- 17β-sulphate by liquid chromatography/tandem mass spectrometry (LC/MS/ MS) had potential for the detection of 19-nortestosterone misuse in the male horse. The high concentrations of sulphate conjugates of the female sex hormones naturally present in male equine urine were overcome by selective hydrolysis of the aryl sulphates using glucuronidase from *Helix pomatia*; this was shown to have little or no activity for alkyl sulphates such as 19-nortestosterone-17β-sulphate. The 'free' phenolic steroids were removed by solid-phase extraction (SPE) prior to LC/MS/MS analysis. The method also allowed for the quantification of the sulphate conjugate of boldenone, a further anabolic steroid endogenous in the male equine with potential for abuse in sports. The method was applied to the quantification of these analytes in a population of samples. This paper reports the results of that study along with the development and validation of the LC/MS/MS method. The results indicate that while 19-nortestosterone-17β-sulphate is present at low levels as an endogenous substance in urine from 'normal' male horses, its use as an effective threshold substance may be viable

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Anal Chem 2008 **80** (10) 3811
Differentiation and identification of recombinant human erythropoietin and darbepoetin in equine plasma by LC-MS/MS for doping control
The stimulation of erythrocyte production with protein-based drugs such as recombinant human erythropoietin (rhEPO) and darbepoetin α (DPO) is employed to treat anemia in humans. These agents have been abused in human and equine sports due to their potential to enhance performance. The differentiation and identification of rhEPO and DPO in equine plasma by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) id described herein. Immunoaffinity separation with anti-rhEPO antibodies, dual digestion by trypsin and peptide-N-glycosidase F (PNGase F), and analysis by LC-MS/MS was employed to extract and enrich the analyte. Two unique deglycosylated tryptic peptides, ^{21}EAENITTGCAEHCSLNENITVPDTK^{45} (T_5) from rhEPO and ^{77}GQALLVNSSQVNETLQLHVDK^{97} (T_9) from DPO, were employed for differentiation and identification of rhEPO and DPO *via* LC retention times and major product ions. The limit of identification was 0.1 ng/ml for DPO and 0.2 ng/ml for rhEPO in equine plasma, and the limit of detection was 0.05 ng/ml for DPO and 0.1 ng/ml for rhEPO. A problem with analyte carryover was encountered and solved by adding 20% acetonitrile to the solvent of the sample digest to increase solubility of the peptides. This technique was successfully employed for identification of DPO in plasma samples collected from a research horse following DPO administration and from racehorses out of competition in North America. Consequently, it provides a powerful tool in the fight against blood doping with rhEPO and DPO in the horse racing industry

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J Mass Spectrom 2008 **43** (9) 1255
Direct injection LC/ESI-MS horse urine analysis for the quantification and identification of threshold substances for doping control. I. Determination of hydrocortisone
Two simple and rapid LC/MS methods with direct injection analysis were developed and validated for the quantification and identification of hydrocortisone in equine urine using the same sample preparation but different mass spectrometric systems: ion trap mass spectrometry (IT-MS) and time-of-flight mass spectrometry (TOF-MS). The main advantage of the proposed methodology is the minimal sample preparation procedure, as particle-free diluted urine samples were directly injected into both LC/MS systems. Desonide was used as internal standard (IS). The linear range was 0.25-2.5 µg/ml for both methods. Matrix effects were evaluated by preparing and analyzing calibration curves in water solutions and different horse urine samples. A great variation of the signal both for hydrocortisone and the internal standard was observed in different matrices. To overcome matrix effects, the unavailability of blank matrix and the excessive cost of the isotopically labeled internal standard, standard additions calibration method was applied. This work is an exploration of the performance of the standard additions approach in a method where neither nonisotopic internal standards nor extensive sample preparation is utilized and no blank matrix is available. The relative standard deviations of intra and interday analysis of hydrocortisone in horse urine were lower than 10.2 and 5.4%, respectively, for the LC/IT-MS method and lower than 8.4 and 4.4%, respectively, for the LC/TOF-MS method. Accuracy (bias percentage) was less than 9.7% for both methods

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Anal Sci 2008 **24** (7) 911
Characterization and quantification of fluoxymesterone metabolite in horse urine by gas chromatography/mass spectrometry
Fluoxymesterone is an anabolic steroid with the 17α-methyl,17β-hydroxy group. It has been developed as an oral formulation for therapeutic purposes. However, it is also used illegally in racehorses to enhance racing performance. In this study, we detected 9α-fluoro-17,17-dimethyl-18-norandrostane-4,13-dien-11β-ol-3-one by gas chromatography/mass spectrometry (GC/MS), which has not been reported as a fluoxymesterone metabolite so far in horse. It was synthesized for use as a reference standard, and characterized on the basis of ^{1}H NMR and ^{13}C NMR spectra, as well as GC/MS EI mass spectra of TMS derivatives. It was excreted as the main metabolite in horse urine, and its reference standard could be synthesized easily. Therefore, this metabolite could be a useful marker in a doping test of fluoxymesterone in racehorses

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Anal Sci 2008 **24** (9) 1199

Simultaneous doping analysis of main urinary metabolites of anabolic steroids in horse by ion-trap gas chromatography-tandem mass spectrometry

The use of anabolic steroids in racehorses is strictly regulated. A method has been developed for the simultaneous analysis of 11 anabolic steroids: fluoxymesterone, 17α-methyltestosterone, mestanolone, methandienone, methandriol, oxymetholone, boldenone, furazabol, methenolone, nandrolone, and stanozolol with the possible application in respect of racehorses. Fifteen types of target substances were selected for a doping test from the main metabolites of anabolic steroids, and a method was established for their simultaneous analysis. Urine was hydrolyzed and subjected to solid-phase extraction. Subsequently, the residue from the extracts was derivatized by trimethylsilylation. These were subjected to ion-trap gas chromatography-tandem mass spectrometry and their mass chromatograms and product ion spectra were obtained. The limit of detection of the target substances was 5-50 ng/mL, and the mean recovery and coefficient of variation were 71.3-104.8% and 1.1-9.5%, respectively

8 Recreational Drugs - General

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Anal Bioanal Chem 2008 **391** (6) 2329

Determination of illicit and medicinal drugs and their metabolites in oral fluid and preserved oral fluid by liquid chromatography-tandem mass spectrometry

Oral fluid (0.5 ml) was analyzed for morphine, codeine, 6-monoacetylmorphine, methadone, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxy-*N*-ethylamphetamine, benzoylecgonine, cocaine, delta-9-tetrahydrocannabinol, zolpidem, zopiclone, alprazolam, clonazepam, oxazepam, nordiazepam, lorazepam, flunitrazepam, diazepam, diphenhydramine and amitriptyline using an LC-MS/MS method. The method was fully validated in terms of linearity (the method was linear between 1-5 µg/l and 100-200 µg/l) recoveries (7.5-82.6%), within-day and between-day precisions and accuracies (CV and MRE, both <15%), limits of detection (0.5 µg/l) and quantitation (the lowest point on the calibration curve), relative ion intensities, freeze-and-thaw stability and matrix effect. The technique was employed with preserved oral fluid collected by a special commercial device, the StatSure Saliva Sampler

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J Anal Toxicol 2008 **32** (5) 364

Drug screening of hair by liquid chromatography-tandem mass spectrometry

In addition to blood and urine, hair has become an important matrix for drug analysis. A prolonged detection period makes hair analysis suitable for the detection of exposure to illegal and medicinal drugs for periods up to 12 months. A liquid chromatography-tandem mass spectrometry (LC-MS-MS) technique for drug screening in hair has been developed and validated. To 20 mg of hair, 0.45 ml of acetonitrile/25 mM formic acid (5:95 v/v) and 50 microL of deuterated internal standards were added, and the sample was incubated in a water bath at 37 degrees C for 18 h. LC separation was achieved with a Zorbax SB-Phenyl column (2.1 x 100 mm, 3.5-µm particle). Mass detection was performed by positive ion mode electrospray LC-MS-MS and included the following drugs/metabolites: nicotine, cotinine, morphine, 6-monoacetylmorphine, codeine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, cocaine, benzoylecgonine, 7-aminonitrazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, oxazepam, diazepam, alprazolam, zopiclone, zolpidem, carisoprodol, meprobamate, buprenorphine, and methadone. Within- and between-assay relative standard deviations varied from 2.0% to 12% and 2.7% to 15%, respectively. The accuracies were in the range of -24% to 16%, and recoveries ranged from 25% to 100%. The LC-MS-MS technique proved to be simple and robust for the determination of drugs in hair. It has been used for authentic samples in our laboratory in the past year

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Rapid Commun Mass Spectrom 2008 **22** (7) 979

Direct analysis of illicit drugs by desorption atmospheric pressure photoionization

The feasibility of desorption atmospheric pressure photoionization (DAPPI) in the direct analysis of illicit drugs was demonstrated by the analysis of confiscated drug samples of various forms such as tablets, blotter paper, and plant resin and bloom. 3,4-Methylenedioxymethamphetamine (MDMA), amphetamine, phenazepam, and buprenorphine were detected from the analyzed tablets, lysergic acid diethylamide (LSD) and bromobenzodifuranylisopropylamine (bromo-Dragonfly, ABDF) from blotter paper, and Δ^9-tetrahydrocannabinol (THC) and cannabinol from Cannabis Sativa bloom and resin. The amphetamines, phenazepam and ABDF showed protonated molecules independent of the solvent used, whereas buprenorphine, LSD and the cannabinoids showed molecular ions with toluene and protonated molecules with acetone as the solvent

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Yakugaku Zasshi 2008 **128** (6) 971

Analytical data of designated substances (Shitei-Yakubutsu) controlled by the pharmaceutical affairs law in Japan, part I: GC-MS and LC-MS

Over the last decade, many analogs of narcotic substances have been widely distributed in Japan as readily available psychotropic substances and this has become of great concern. They have been marketed as video cleaners, incense and reagents via the Internet or in video shops. They are not controlled under the Narcotics and Psychotropics Control Law because their pharmacological effects have not yet been proved scientifically. Legislation to inhibit the abuse of these substances was enacted by the Ministry of Health, Labor and Welfare which amended the Pharmaceutical Affairs Law in 2006 so that 31 non-controlled psychotropic substances (11 tryptamines, 11 phenethylamines, 6 alkyl nitrites, 2 piperazines and salvinorin A) and 1 plant (*Salvia divinorum*) are now controlled as "Designated Substances (Shitei-Yakubutsu)" as of April 2007. A further five compounds (4 phenethylamines and 1 piperazine) were added to this category in January 2008. In this study, we have developed simultaneous analytical methods for these designated substances using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) and present retention times, UV spectra, electron ionization (EI), GC-MS, and electrospray ionization (ESI) LC-MS data

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Electrophoresis 2008 **29** (10) 2193

Validation of chiral capillary electrophoresis-electrospray ionization-mass spectrometry methods for ecstasy and methadone in plasma

Due to its selectivity and sensitivity, CE coupled to MS (CE-MS) has evolved as a useful analytical tool for determining drugs and metabolites in biological samples. A generic CE-ESI/MS method was developed for the enantioselective determination of basic compounds in plasma. The use of protein precipitation (PP) prior to a hydrodynamic injection (HD) was well adapted to high-concentration samples (>1 ppm) and allowed high throughput. In contrast, the combination of liquid-liquid extraction (LLE) and electrokinetic injection (EK) was time-consuming but did allow detection at the ppb level. Both approaches were fully validated according to ICH guidelines and SFSTP protocols for two pharmaceutical compounds (ecstasy and methadone (MTD)). Deuterated internal standards (IS) in the analytical procedures were used and good quantitative performance was obtained in terms of trueness and precision (repeatability and intermediate precision) since accuracy profiles were within the acceptance limits (30% for biological assay). Methods were linear over the concentration range of 0.50-175 ng/ml and 0.25-5 µg/ml for LLE-EK and PP-HD procedures, respectively. The LLE-EK methodology was finally successfully applied to quantitation of ecstasy and MTD in real cases obtained from toxicology

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Yakugaku Zasshi 2008 **128** (6) 981

Analytical data of designated substances (Shitei-Yakubutsu) controlled by the pharmaceutical affairs law in Japan, part II: Color test and TLC

The Internet provides ready access to many psychotropic substances in Japan. To inhibit the spread of drug abuse, some compounds have been controlled as designated substances (Shitei-Yakubutsu) in Japan since 2007 by the Pharmaceutical Affairs Law. Twenty-nine designated substances (classified as tryptamine, phenethylamine and piperazine types) were analyzed using color tests and TLC. The color tests were examined with the Marquis, Ehrlich, Simon's, Liebermann-Burehard's, and Mandelin reagents. The color of beta-carbonyl-methylenedioxyphenetylamines produced by the Marquis reagent was yellow, and 4-halo-2,5-dimethoxy phenethylamines reacted with the Marquis reagent to a give deep yellow-green and/or a deep green color. Whereas all designated substances of the tryptamine type reacted with the Ehrlich reagent to give a brown color, only 1-(2,4,6-trimethoxyphenyl) propan-2-amine (TMA-6) among the phenethylamines showed a red color on treatment with the reagent. However, 3,4,5-trimethoxy and 2,4,5-trimethoxy isomers of TMA-6 were not colored with the reagent. Therefore, TMA-6 could be distinguished from isomers using the Ehrlich reagent. Designated substances were also analyzed with thin-layer chromatography developed with two different

solvent conditions. All substances were detected by UV(254 nm) and an iodoplatinate reagent. These results suggest that color tests and TLC, followed by GC-MS and LC-MS analyses, may be used for preliminary identification of designated substances

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Rapid Commun Mass Spectrom 2008 **22** (6) 887

Simultaneous quantitative determination of amphetamines, ketamine, opiates and metabolites in human hair by gas chromatography/mass spectrometry

A gas chromatography/mass spectrometry (GC/MS) method was developed and validated for the determination of common drugs of abuse in Asia. The method was able to simultaneously quantify amphetamines (amphetamine; AP, methamphetamine; MA, methylenedioxy amphetamine; MDA, methylenedioxymethamphetamine; MDMA, methylenedioxy ethylamphetamine; MDEA), ketamine (ketamine; K, norketamine; NK), and opiates (morphine; MOR, codeine; COD, 6-acetylmorphine; 6-AM) in human hair. Hair samples (25 mg) were washed, cut, and incubated overnight at 25°C in methanol/trifluoroacetic acid (methanol/TFA). The samples were extracted by solid-phase extraction (SPE), derivatized using heptafluorobutyric acid anhydride (HFBA) at 70°C for 30 min, and the derivatives were analyzed by electron ionization (EI) GC/MS in selected ion monitoring mode. Confirmation was accomplished by comparing retention times and the relative abundances of selected ions with those of standards. Deuterated analogs of the analytes were used as internal standards for quantification. Calibration curves for ten analytes were established in the concentration range 0.1-10 ng/mg with high correlation coefficients ($r^2 > 0.999$). The intra-day and inter-day precisions were within 12.1% and 15.8%, respectively. The intra-day and inter-day accuracies were between -8.7% and 10.7%, and between -5.9% and 13.8%, respectively. The limit of detection (LOD) and limit of quantification (LOQ) obtained were 0.03 and 0.05 ng/mg for AP, MA, MDA, MDMA and MDEA; 0.05 and 0.08 ng/mg for K, NK, MOR and COD; and 0.08 and 0.1 ng/mg for 6-AM. The recoveries were above 88.6% for all the compounds, except K and NK which were in the range of 71.7-72.7%. Eight hair samples from known polydrug abusers were examined by this method. These results show that the method is suitable for broad-spectrum drug testing in a single hair specimen

9 Stimulants

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Rapid Commun Mass Spectrom 2008 **22** (20) 3320

Development and validation of an analytical method for the simultaneous determination of cocaine and its main metabolite, benzoylecgonine, in human hair by gas chromatography/mass spectrometry

A new, simple and rapid procedure has been developed and validated for the determination of cocaine and its main metabolite, benzoylecgonine, in human hair samples. After extraction from within the hair matrix by a mixture of methanol/hydrochloric acid (2:1) at 65°C for 3 h, and sample cleanup by mixed-mode solid-phase extraction (SPE), the extracts were analyzed by gas chromatography/mass spectrometry (GC/MS), after derivatization with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide with 5% chlorotrimethylsilane. Using a sample size of only 20 mg of hair, limits of detection (LODs) and quantitation (LOQs) were, respectively, 20 and 50 pg/mg for cocaine, and 15 and 50 pg/mg for benzoylecgonine, achieving the cut-off values proposed by the Society of Hair Testing for the analysis of these compounds in hair. The method was found to be linear (weighing factor of 1/x) between the LOQ and 20 ng/mg for both compounds, with correlation coefficients ranging from 0.9974 to 0.9996 for cocaine; and from 0.9981 to 0.9994 for benzoylecgonine. Intra- and interday precision and accuracy were in conformity with the criteria normally accepted in bioanalytical method validation. The sample cleanup step presented a mean absolute recovery greater than 90% for both compounds. The developed method may be useful in forensic toxicology laboratories for the analysis of cocaine and benzoylecgonine in hair samples, taking into account its speed (only 3 h are required for the extraction of the analytes from within the matrix, whereas 5 h or even overnight extractions have been reported) and the low limits achieved (using a single quadrupole mass spectrometer, which is available in most laboratories)

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Anal Bioanal Chem 2008 **391** (4) 1309

Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry

Solid-phase extraction (SPE) and liquid chromatography-ion trap tandem mass spectrometry (LC-MS/MS) has been employed and validated for the determination of cocaine (COC) and its principal metabolites, benzoylecgonine (BE) and ecgonine methyl ester (EME), in waste and surface water. Several SPE adsorbents were investigated and the highest recoveries (95.7 +/- 5.5, 91.8 +/- 2.2 and 72.5 +/- 5.3% for COC, BE and EME, respectively) were obtained for OASIS HLB® cartridges (6 ml/500 mg) using 100 ml of waste water or 500 ml of surface water. Extracts were analysed by reversed-phase (RP) or hydrophilic interaction (HILIC) LC-MS/MS in positive ion mode with multiple reactions monitoring (MRM); the latter is the first reported application of the HILIC technique for drugs of abuse in water samples. Deuterated internal standards of compounds were used for quantification. The method limits of quantification (LOQs) for COC and BE were 4 and 2 ng/l, respectively, when RPLC was used and 1, 0.5 and 20 ng/l for COC, BE and EME, respectively, with the HILIC setup. For COC and BE, the LOQs were below the concentrations measured in real water samples. Stability tests were conducted to establish the optimal conditions for sample storage (pH, temperature and time). The degradation of COC was minimal at -20°C and pH = 2, but it was substantial at +20°C and pH = 6. A set of waste and surface water samples collected in Belgium were analyzed by this validated method

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J Sep Sci 2008 **31** (13) 2444

An accurate and nondestructive GC method for determination of cocaine on US paper currency

The presence of cocaine on US paper currency has been known for a long time. Banknotes become contaminated during the exchange, storage, and abuse of cocaine. The analysis of cocaine on various denominations of US banknotes in the general circulation can provide law enforcement circles and forensic epidemiologists objective and timely information on epidemiology of illicit drug use and on how to differentiate money contaminated in the general circulation from banknotes used in drug transaction. A simple, nondestructive, and accurate capillary gas chromatographic method has been developed for the determination of cocaine on various denominations of US banknotes in this study. The method comprises a fast ultrasonic extraction using water as a solvent followed by a SPE cleanup process with a C_{18} cartridge and capillary GC separation, identification, and quantification. This nondestructive analytical method has been successfully applied to determine the cocaine contamination in US paper currency of all denominations. Standard calibration curve was linear over the concentration range from the LOQ (2.00 ng/ml) to 100 μg/ml and the RSD less than 2.0%. Cocaine was detected in 67% of the circulated banknotes collected in Southeastern Massachusetts in amounts ranging from approximately 2 ng to 49.4 μg per note. On average, $5, 10, 20, and 50 denominations contain higher amounts of cocaine than $1 and 100 denominations of US banknotes

10 Hallucinogens

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J Chromatogr Sci 2008 **46** (8) 671

GC-MS analysis of ring and side chain regiosomers of ethoxyphenethylamines

3,4-Methylenedioxymethamphetamine (3,4-MDMA), a controlled drug, and its 2,3-regioisomer were differentiated from the ring substituted ethoxyphenethylamines by mass spectroscopy after formation of the perfluoroacyl derivatives, pentafluoropropionamides (PFPA), and heptafluorobutyrylamides (HFBA). The ring substituted ethoxyphenethylamines constitute a unique set of compounds having an isobaric relationship with 3,4-MDMA. These isomeric forms of the 2-, 3-, and 4-ethoxy phenethylamines have mass spectra essentially equivalent to 3,4-MDMA; all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. All the side chain regioisomers of 2-ethoxy phenethylamine having equivalent mass spectra to 3,4-MDMA are synthesized and compared *via* gas chromatography-mass spectrometry to 2,3- and 3,4-methylenedioxymethamphetamine. The mass spectra for the perfluoroacyl derivatives of the primary and secondary amine regioisomers are significant entities, and the side chain regioisomers yield unique hydrocarbon fragment ions at *m/z* 148, 162, and 176. Furthermore, the substituted ethoxymethamphetamines are distinguished from the methylenedioxymethamphetamines via the presence of the *m/z* 107 ion. Gas chromatographic separation on relatively non-polar stationary phases successfully resolves these derivatives

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Anal Chem 2008 **80** (9) 3350

Emerging use of isotope ratio mass spectrometry as a tool for discrimination of 3,4-methylenedioxymethamphetamine by synthetic route

An aspiration of law enforcement agencies has long been drug profiling, or the ability to link batches of illicit drugs to a common source or synthetic route. Drug profiling with isotope ratio mass spectrometry (IRMS) has been explored in the past decade. Research has been limited by the use of substances seized by police where the provenance is unknown. However, recent studies have been carried out on drugs synthesized in-house and therefore of known history. In this study, 18 MDMA samples were synthesized in-house from aliquots of the same precursor by three common reductive amination routes and analyzed for ^{13}C, ^{15}N, and ^{2}H isotope abundance using IRMS. In these three preparative methods, results indicate that ^{2}H isotope abundance data is necessary for discrimination by synthetic route. Moreover, hierarchical cluster analysis using ^{2}H data on its own or combined with ^{13}C and/or ^{15}N provides a statistical means for accurate discrimination by synthetic route

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J Anal Toxicol 2008 **32** (4) 298

A rapid GC-MS determination of -hydroxybutyrate in saliva

The forensic toxicology of GHB and its related compounds has been investigated for many years due to their illicit use in drug-facilitated sexual assault, and to a lesser extent, as party drugs. The identification and quantification of gamma-hydroxybutyric acid (GHB) in saliva by a sensitive and specific method has been developed using gas chromatography-mass spectrometry with selective ion monitoring mode. One microliter of synthetic saliva was spiked with 1.0 µl of GHB-d_6 as the internal standard and 1.0 µl of 1,7-heptanediol as a surrogate spike to all samples. After a silyl-derivatization, the sample was injected at a split ratio of 10:1. The following ions were monitoring: 233 and 234 for GHB; 239, 240, and 241 for GHB-d_6; and 55, 73, and 97 for 1,7-heptanediol. The limit of quantitation was determined to be 0.5 mg/l with a linear dynamic range of 0.5-50.0 mg/l. Quality control samples (5.0, 20.0, and 30.0 mg/l) were prepared for the evaluation of precision. Analytical precision measured by coefficients of variation ranged from 2.1% to 12.50% in both intraday and day-to-day experiments. Surrogate recovery from saliva samples fell in the range of 94.6% to 100% with an average of 98.37% and a corresponding percent relative standard deviation of 1.2%

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Anal Bioanal Chem 2008 **390** (7) 1837

Metabolism and toxicological detection of the designer drug 4-chloro-2,5-dimethoxyamphetamine in rat urine using gas chromatography-mass spectrometry

The metabolism and the toxicological analysis of the amphetamine-derived designer drug 4-chloro-2,5-dimethoxyamphetamine (DOC) was investigated in rat urine using gas chromatographic-mass spectrometric techniques. Metabolites identified suggested that DOC was metabolized by *O*-demethylation at position 2 or 5 of the phenyl ring partly followed by glucuronidation and/or sulfation. A systematic toxicological analysis procedure using full-scan gas chromatography-mass spectrometry after acid hydrolysis, liquid-liquid extraction and microwave-assisted acetylation allowed the detection of an intake of a dose of DOC in rat urine that corresponds to a common drug user's dose. The STA procedure described should be suitable as proof of an intake of DOC in human urine providing that metabolism is similar

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J Mass Spectrom 2008 **43** (5) 674

Isomerization of -9-THC to -8-THC when tested as trifluoroacetyl-, pentafluoropropionyl-, or heptafluorobutyryl- derivatives

For GC-MS analysis of delta-9-tetrahydrocannabinol (Δ-9-THC), perfluoroacid anhydrides in combination with perfluoroalcohols are commonly used for derivatization. This reagent mixture is preferred because it allows simultaneous derivatization of Δ-9-THC and its acid metabolite, 11-nor-Δ-9-THC-9-carboxylic acid present in biological samples. When Δ-9-THC was derivatized by trifluoroacetic anhydride/hexafluoroisopropanol (TFAA/HFIPOH) and analyzed by GC-MS using full scan mode (50-550 amu), two peaks (P1 and P2) with an identical molecular mass of 410 amu were observed. On the basis of the total ion chromatogram (TIC), P1 with a shorter retention time (RT) was the major peak (TIC 84%). To identify the peaks, Δ-8-THC was also tested under the same conditions. The RT and spectra of the major peak (TIC 95%) were identical with that of P1 for Δ-9-THC. A minor peak (5%) present also correlated well with the latter peak (P2) for the Δ-9-THC derivative. The fragmentation pathway of P1 was primarily demethylation followed by retro Diels-Alder fragmentation (M - 15-68, base peak 100%) indicating P1 as a Δ-8-THC-trifluoroacetyl compound. This indicated that Δ-9-THC isomerized to Δ-8-THC during derivatization with TFAA/HFIPOH. Similar results were also observed when Δ-9-THC was derivatized with pentafluoropropionic anhydride/pentafluoropropanol or heptafluorobutyric anhydride/heptafluorobutanol. No isomerization was observed when chloroform was used in derivatization with TFAA. In this reaction, the peaks of Δ-8-THC-TFA and Δ-9-THC-TFA had retention times and mass spectra matching with P1 and P2, respectively. Because of isomerization, perfluoroacid anhydrides/perfluoroalcohols are not suitable derivatizing agents for analysis of Δ-9-THC; whereas the TFAA in chloroform is suitable for the analysis

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J Anal Toxicol 2008 **32** (5) 349

Determination of cannabinoids in whole blood by UPLC-MS-MS

The simultaneous determination of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in whole blood has been analyzed by the development and validation of a technique employing ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS-MS). Protein precipitation followed by solid-phase extraction was used to prepare samples. Positive electrospray ionization and multiple reaction monitoring provided the data. Two transitions were selected for THC (m/z 315.0 > 193.0 and m/z 315.0 > 122.7) and THC-COOH (m/z 345.0 > 299.0 and m/z 345.0 > 327.0), and one transition was chosen for 11-OH-THC (m/z 331.0 > 313.0). Deuterated analogues of each analyte were used as internal standards for quantification. Run time was 10 min. Limits of quantification (LOQ) were 0.05 ng/ml for THC, 0.1 ng/ml for 11-OH-THC, and 0.2 ng/ml for THC-COOH. Linearity was established from LOQ to 50 ng/ml for each substance (r^2 always > 0.999). Accuracy ranged from 96 to 106%, and imprecision was less than 10% for all analytes. Since it requires no derivatization step, the UPLC-MS-MS method was found to be sensitive, specific, and rapid. It can be an alternative to gas chromatography-mass spectrometry for the determination of cannabinoids in whole blood

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J Anal Toxicol 2008 **32** (6) 417

The detection and quantitative analysis of the psychoactive component of *Salvia divinorum*, salvinorin A, in human biological fluids using liquid chromatography-mass spectrometry

Recreational drug users have increasingly sought , a member of the mint plant family which has hallucinogenic properties. Salvinorin A is the main psychoactive component with a potency comparable to lysergic acid diethylamide. Whereas it is still legal to possess salvinorin in most of the United States and much of Europe, little is known regarding the compound's long-term health effects, addiction liability, and pharmacokinetics. The scientific literature contains limited data and few analytical methods are published for the detection in human biological fluids. These factors contribute to the unfamiliarity of the compound and complicate the method development process necessary to provide special requested testing for salvinorin A. A sensitive analytical technique for the detection and quantitation of salvinorin A in human biological fluids has been developed and validated to resolve analytical shortcomings. The method employs a solid-phase extraction technique coupled with liquid chromatography-electrospray ionization mass spectrometry operated in selected ion monitoring mode. The assay has a linear range of 5.0-100 ng/ml with a correlation coefficient of 0.997. The limit of detection and limit of quantitation were experimentally determined as 2.5 and 5.0 ng/ml, respectively. Blood and urine samples have been analyzed successfully and the method may be used to detect the presence of salvinorin A in forensic testing

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J Mass Spectrom 2008 **43** (4) 528

Discrimination and identification of the six aromatic positional isomers of trimethoxyamphetamine (TMA) by gas chromatography-mass spectrometry (GC-MS)

A reliable and accurate GC-MS method was developed that allows both mass spectrometric and chromatographic discrimination of the six aromatic positional isomers of trimethoxyamphetamine (TMA). Regardless of the trifluoroacetyl (TFA) derivatization, chromatographic separation of all the investigated isomers was achieved by using DB-5 ms capillary columns (30 m x 0.32 mm i.d.), with run times less than 15 min. However, the mass spectra of the nonderivatized TMAs, except 2,4,6-trimethoxyamphetamine (TMA-6), showed insufficient difference for unambiguous discrimination. On the other hand, the mass spectra of the TFA derivatives of the six isomers exhibited fragments with significant intensity differences, which allowed the unequivocal identification of all the aromatic positional isomers investigated in the present study. This GC-MS technique in combination with TFA derivatization, therefore, is a powerful method to discriminate these isomers, especially useful to distinguish the currently controlled 3,4,5-trimethoxyamphetamine (TMA-1) and 2,4,5-trimethoxyamphetmine (TMA-2) from other uncontrolled TMAs

11 Narcotics

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Electrophoresis 2008 **29** (4) 936

Pressure-assisted capillary electrochromatography with electrospray ionization-mass spectrometry based on silica-based monolithic column for rapid analysis of narcotics

A pressure-assisted CEC (pCEC) with ESI-MS based on silica-based monolithic column was developed for rapid analysis of narcotics. Combining the extremely high permeability and separation efficiency of silica-based monolithic column with the high selectivity and sensitivity of pCEC-ESI-MS, the developed system exhibited its prominent advantages in separation and detection. A systematic investigation of the pCEC separation and ESI-MS detection parameters was performed. Experiment results showed that the optimized separation efficiency could be obtained at 8 bar assisted pressure with 25 kV separation voltage, using the solution containing 65% ACN v/v and 20 mmol/l ammonium acetate with pH 6.0 as running buffer. 3 µl/min of sheath liquid was considered as the optimized flow rate since it could provide the maximum signal intensity. Under the optimum conditions, the tested five narcotics could be completely separated within 10 min with the detection limit in the range of 2.0-80 nmol/l. The proposed method has been successfully used for detection of narcotics in real urine samples

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Rapid Commun Mass Spectrom 2008 **22** (18) 2851

Fragmentation pathways of heroin-related alkaloids revealed by ion trap and quadrupole time-of-flight tandem mass spectrometry

The electrospray ionization (ESI) ion trap and quadrupole time-of-flight (QqToF) mass spectra of heroin and seven related alkaloids, i.e., morphine, codeine, *O*-6-monoacetylmorphine (6-MAM), thebaine, acetylcodeine, papaverine and narcotine, have been extensively investigated in this work. The ESI mass spectrometric fragmentation pathways of protonated 6-MAM, heroin, acetylcodeine, and thebaine were comprehensively elucidated for the first time with the aid of high-resolution mass spectrometry. It was found that cleavage of the piperidine ring was the featured fragmentation route of six of the compounds, although not of papaverine and narcotine. In addition, a simple high-performance liquid chromatography (HPLC)-based separation method gave baseline resolution of all eight components. This study could play an important role in the screening for these alkaloids in different matrices by HPLC coupled to tandem mass spectrometry (MS/MS)

12 Forensics

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Rapid Commun Mass Spectrom 2008 **22** (12) 1965

Succinylmonocholine analytics as an example for selectivity problems in high-performance liquid chromatography/tandem mass spectrometry, and resulting implications for analytical toxicology

The determination and quantitation of drugs in biological matrices using high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) is becoming increasingly popular in analytical toxicology, while at the same time a growing awareness for the limits of this technique can be observed. Our group previously developed a rapid HPLC/ESI-MS/MS method for the detection and quantitation of succinylcholine (SUX) and succinylmonocholine (SMC) using ion-pairing extraction of samples with subsequent separation by gradient chromatography on a Synergi Hydro RP C_{18} column (4 µm, 150 x 2 mm). Identification of analytes was achieved in the multiple reaction monitoring (MRM) mode, using two characteristic ion transitions each, the respective analytes' retention time as well as co-elution of stable isotopic analogues. In both native serum as well as urine an interference with the main MRM transition of SMC was found to co-elute with this analyte, thus severely compromising the identification and quantitation of this target analyte. The interference was further shown to be eliminated from serum and urine by exposure to alkaline conditions and hence proven to share a key physicochemical property with SMC. The observed absence of the second and third most intense ion transitions of SMC in the unknown substance was the only useful distinction between both compounds.The detailed presentation of selectivity problems encountered during method development is intended to initiate further discussion on this yet underrepresented issue in HPLC/MS/MS. The present work emphasizes the need to monitor more than just one ion transition to confidently rule out signal interferences, ensure correct analyte identification as well as quantitation, and thus avoid false-positive results. In this context, the employment of minor MRM transitions for the quantitation and identification of a given analyte is presented as a satisfactory solution to HPLC/MS/MS selectivity problems, and proposed as a possible alternative to previously published approaches

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J Mass Spectrom 2008 **43** (10) 1344

A fully validated isotope dilution HPLC-MS/MS method for the simultaneous determination of succinylcholine and succinylmonocholine in serum and urine samples

A high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) method for the simultaneous detection of succinylcholine (SUX) and its metabolite succinylmonocholine (SMC) in serum and urine is presented. For internal standardization using isotope dilution, the deuterated compounds SUX-d_{18} and SMC-d_3 were employed. Full validation was performed according to international guidelines. Solid-phase extraction (SPE) of acidified samples was accomplished using Strata-X polymeric reversed phase cartridges together with heptafluorobutyric acid (HFBA) as ion-pairing reagent. Separation was achieved within 13 min on a Phenomenex Synergi Hydro RP C_{18} column (4 µm, 150 x 2 mm) using a gradient of 5 mM ammonium formate buffer pH 3.5 and acetonitrile. To ensure the method's applicability in forensic as well as clinical toxicology, the specific demands of both research fields were taken into account, and the method was thus validated for a low and high concentration range. For both serum and urine as sample matrix, the validation revealed good intraday and interday precisions, consistently ranging below 15% for the lowest and below 10% for elevated concentrations. Accuracy was likewise good and never exceeded 10%. Extraction recovery was excellent, ranging between 88.1 and 103.9% for SUX and SMC in both tested matrices. Matrix effects were significant, the otherwise optimized extraction and detection methods, however, allowed for a very satisfactory sensitivity of the described method: For serum, the limits of detection and quantitation were determined to be 1.9 and 6.0 ng/ml for SUX, as well as 2.5 and 8.6 ng/ml for SMC, respectively; for urine, the corresponding values were established to be 1.4 and 4.0 ng/ml (SUX), as well as 1.5 and 4.9 ng/ml (SMC).The presented method was successfully applied to authentic samples of two forensic cases investigated in the institute of forensic medicine in Bonn, allowing the diagnosis of SUX intoxications

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J Sep Sci 2008 **31** (12) 2265

Determination of tricyclic antidepressants in human plasma using pipette tip solid-phase extraction and gas chromatography-mass spectrometry

A method for the simultaneous extraction of four tricyclic antidepressants from human plasma samples using pipette tip SPE with MonoTip C_{18} tips is presented. Human plasma (0.1 ml) containing four tricyclic antidepressants (amitriptyline, amoxapine, imipramine, and trimipramine) and an internal standard (IS), protriptyline, was mixed with 0.4 ml of distilled water and 100 microL 1 M NaOH solution. After centrifugation of the mixture, the supernatant was extracted to the C_{18} phase of the tip by 20 repeated aspirating/dispensing cycles using a manual micropipettor. The analytes retained in the tip were eluted with methanol by five repeated aspirating/dispensing cycles. Without evaporation and reconstitution, the eluate was directly injected into a gas chromatograph injector and detected by a mass spectrometer with SIM in the positive-ion electron impact mode. Recovery of the four antidepressants and IS spiked into human plasma was 80.2-92.1%. The regression equations for the four antidepressants showed excellent linearity in the range of 0.2-40 ng/0.1 ml. LODs and LOQs for the four drugs were 0.05-0.2 ng/0.1 ml and 0.2-0.5 ng/0.1 ml, respectively. Intra- and interday CVs for the four drugs in plasma were no greater than 9.5%

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Anal Chem 2008 **80** (8) 3050

Implementation and performance evaluation of a database of chemical formulas for the screening of pharmaco/toxicologically relavent compounds in biological samples using electrospray ionization-time-of-flight mass spectrometry

A wide range of pharmaco/toxicologically relevant compounds (PTRC) in biosamples may be analysed by electrospray ionization (ESI)-time-of-flight (TOF) MS. The number of identifiable PTRC require an expansion of the reference database of chemical formulas/compound names. Previous in-house or commercial databases with limitations either in PTRC number or content have been suggested (e.g., few metabolites, presence of non-PTRC). In the context of the development of a ESI-TOF PTRC screening procedure, a subset of PubChem Compound as reference database is proposed. Parameters of this database (approximately 50,500 compounds) are illustrated, and its performance evaluated through analysis by capillary electrophoresis (CE)-ESI-TOF of hair/blood/urine collected from subjects under treatment with known drugs or by comparison with reference standards. The database is rich in parent

compounds of pharmaceutical and illicit drugs, pesticides, and poisons and contains many metabolites (including about 6000 phase I metabolites and 180 glucuronides) and related substances (e.g., impurities, esters). The average number of hits with identical chemical formula is 1.82 +/- 2.27 (median = 1, range 1-39). Minor deficiencies, redundancies, and errors have been detected that do not limit the potential of the database in identifying unknown PTRC. The database provides for a much broader search for PTRC than other commercial/in-house databases of chemical formulas/compound names previously proposed. However, the chance that a search would retrieve different PTRCs having identical chemical formula is higher than with smaller databases, and additional information (anamnestic/circumstantial data, concomitant presence of parent drug and metabolite, selective sample preparation, liquid chromatographic retention, and CE migration behavior) is required in order to focus the search more tightly

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J Mass Spectrom 2008 **43** (7) 974
Formation of the *N*-methylpyridinium derivative to improve the detection of buprenorphine by liquid chromatography-mass spectrometry
The legally defensible identification of the narcotic, analgesic buprenorphine, in biological specimen requires considerable sensitivity due to its low therapeutic dosages and corresponding target concentrations. Application of liquid chromatography-electrospray ionisation-mass spectrometry, which became the default method for buprenorphine detection, is impeded by the disadvantageous fragmentation of the stable precursor ion producing unspecific product ions of comparatively low abundance. A chemical modification to form the *N*-methylpyridinium ether derivative of buprenorphine is presented to improve the selectivity and sensitivity of its detection by liquid chromatography-mass spectrometry (LC-MS). The reaction of buprenorphine with 2-fluoro-1-methylpyridinium-*p*-toluene-sulfonate and triethylamine as catalyst was accomplished in acetonitrile at an ambient temperature yielding a chemically stable derivative. Fragmentation of the permanently charged precursor ion (*m/z* = 559) leads to the formation of diagnostic and abundant fragments (e.g. *m/z* = 443 and 450) representing all parts of the molecule. The application of the technique to the identification of buprenorphine in hair samples demonstrates a high specificity, availability of sufficient qualifier ions and a significant (approximately 8-fold) improvement of detection limits with respect to comparable experiments based on underivatised buprenorphine

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J Anal Toxicol 2008 **32** (6) 422
Quantitative analysis of the aminosteroidal non-depolarizing neuromuscular blocking agent vercuronium by LC-ESI-MS: A postmortem investigation
In this postmortem investigation, the apparent recreational use of an aminosteroidal non-depolarizing neuromuscular blocking agent, vecuronium, is described. Using liquid chromatography-electrospray ionization mass spectrometry operated in positive selected ion monitoring mode, a quantitative method for the analysis of vecuronium and its active metabolite, 3-desacetylvecuronium, in blood and tissue was developed. Chromatographic separation was performed on a Gemini 5-µm C_{18} column using a mobile phase of 0.1% formic acid/acetonitrile at 0.700 ml/min. The method was linear from 0.01 to 1.00 mg/l with correlation coefficients of 0.999 and greater for both compounds. The limits of detection and quantitation were determined in blood to be 0.005 and 0.010 mg/l, respectively. The coefficients of variation were less than 10% for both intra- and interday assays. Vecuronium was quantitated in blood at 0.070 mg/l and in the kidney, liver, and spleen at 0.224, 0.045, and 0.080 mg/kg, respectively. The active metabolite 3-desacetylvecuronium was quantitated in blood at 0.100 mg/l, in the urine at 0.040 mg/l and in the kidney, liver, spleen, and lung at 0.271, 0.100, 0.082, and 0.164 mg/kg, respectively

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J Anal Toxicol 2008 **32** (6) 444
Detection of 1-benzylpiperazine and 1-(3-trifluoromethylphenyl)-piperazine in urine analysis specimens using GC-MS and LC-ESI-MS
Widely available and popular party drugs globally, are the designer piperazines, such as 1-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)-piperazine (TFMPP). These designer piperazines exhibit several of the same stimulant and psychoactive properties of their illicit counterparts in many countries where they are legal alternatives to methamphetamine and ecstasy. Herein is a case study of seven urine analysis specimens analyzed for designer piperazines. In all seven specimens, a full scan gas chromatography-mass spectrometry screen detected the presence of BZP and TFMPP. Confirmation using liquid chromatography-electrospray ionization-mass spectrometry operating in selected ion monitoring mode (SIM) yielded urinary concentrations ranging from 13.0 to 429.1 mg/l and 0.79 to 25.4 mg/l for BZP and TFMPP, respectively

13 Alcohol

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J Appl Toxicol 2008 **28** (6) 773
Microwave assisted extraction for the determination of ethyl glucuronide in urine by gas chromatography-mass spectrometry
Alcohol is the most frequently abused 'addictive substance' that causes serious social problems throughout the world; thus alcoholism is of particular interest in clinical and forensic medicine. Ethyl glucuronide (EtG) is a marker of recent alcohol consumption that detects alcohol use reliably over a definite time period. The present paper describes a new method for the determination of EtG in urine. It was based both on microwave assisted extraction (MAE) to extract the analyte from urine samples, and gas chromatography-mass spectrometry (GC-MS) to identify and quantify the EtG in selected ion monitoring (SIM) mode. The method was applied to 33 urine samples from alcohol users, obtaining positive results in all cases. It was fully validated including a linear range (0.1-100 µg/ml) and the main precision parameters. In summary, the use of microwave assisted extraction turned out to be a substantially simpler, faster and more sensitive procedure than any other conventional sample preparations

14 Tobacco

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J Chromatogr Sci 2008 **46** (7) 659
Gas chromatographic-mass spectrometric analysis of acrylamide and acetamide in cigarette mainstream smoke after on-column injection
Two short-chained amides, acrylamide and acetamide (classified by the International Agency for Research on Cancer as probable and possible human carcinogens, respectively), have been determined in total particulate matter using gas chromatography-on-column injection and mass spectrometric detection. Sample preparation is minimumized, and the proposed analytical procedure proves to be fast, sensitive, and precise. Validation studies show good linearity with a regression coefficient of r^2=.000 for both compounds. Quantitation limits are 32 ng/ml for acrylamide and 70 ng/ml for acetamide. In the particulate phase of mainstream smoke from the University of Kentucky Reference Cigarette 2R4F, 2.3 µg/cig acrylamide and 4.7 µg/cig acetamide are found; no acetamide and only .0074 µg/cig acrylamide is found in the gas phase. Possible mechanisms of formation in cigarette smoke are discussed

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Anal Bioanal Chem 2008 **391** (8) 2845
Identification of *in vitro* differential cell secretions due to cigarette smoke condensate exposure using nanoflow capillary liquid chromatography and high-resolution mass spectrometry
Cell signaling, growth, and apoptosis are often regulated by secreted proteins, the secretome which may be isolated from biological fluids such as blood. An understanding of disease mechanisms may be derived from the identification of secreted proteins and they can serve as early candidate biomarkers of disease and exposure.However, it is time-consuming and costly to conduct *in vivo* interrogations of the human secretome. To expedite matters, herein is a detailed description of a rapid in vitro technique for the analysis of differential protein secretion due to exposure to smoking-machine-generated cigarette smoke (CS) condensate (total particulate matter, TPM). Endothelial cells were exposed to CS-TPM, the supernatant was collected, and the secretome was elucidated by nano liquid chromatography coupled with high-resolution mass spectrometry. A total of 1,677 unique peptides were identified in the cell culture supernatants. Several proteins were differentially expressed following CS-TPM exposure that relate to several biological processes, such as metabolism, development, communication, response to stimulus, and response to stress

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Rapid Commun Mass Spectrom 2008 **22** (16) 2609
Ultrasensitive detection of nicotine and cotinine in teeth by high-performance liquid chromatography/tandem mass spectrometry (Letter)
No abstract available

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J Chromatogr A 2008 **1198-1199** 27
Development of C_{18}-functionalized magnetic silica nanoparticles as sample preparation technique for the determination of ergosterol in cigarettes by microwave-assisted derivatization and gas chromatography/mass spectrometry
An important precursor of tumorigenic polynuclear aromatic hydrocarbons is

ergosterol. Fungal contamination results in a large amount of ergosterol in mildewy cigarettes. A novel approach is described based on C_{18}-functionalized magnetic silica nanoparticles (C_{18}-f-MS NPs) coupled with microwave-assisted derivatization and gas chromatography/mass spectrometry (GC/MS) for the rapid enrichment and determination of ergosterol in cigarettes. Consequently, microwave-assisted derivatization requires very short time (several minutes), and the extraction and concentration of ergosterol become the key step in the sample preparation process. In this study, the prepared C_{18}-f-MS NPs with its unique properties (high surface area and strong magnetism) provided an efficient way for extraction and concentration of ergosterol in the samples. Furthermore, the analyte of ergosterol adsorbed with C_{18}-f-MS NPs in cigarettes may be simply and rapidly isolated (approx. 2s) through placing a strong magnet on the bottom of container. In this work, different parameters such as added amounts of C_{18}-f-MS NPs, extraction temperature, and extraction time were optimized to enhance the extraction efficiency. Method validations (linear range, detection limit, precision, and recovery) were also examined. The results obtained using the optimal conditions showed that the proposed method based on C_{18}-f-MS NPs was a simple, high efficient, and had a rapid technique for the enrichment of ergosterol in cigarettes. It was successfully applied to the analysis of ergosterol in normal and mildewy cigarettes followed by microwave-assisted derivatization and GC/MS

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J Sep Sci 2008 **31** (13) 2451
Microwave-assisted silylation followed by gas chromatography/mass spectrometry for rapid determination of ergosterol in cigarettes
Ergosterol is one of the important precursors of tumorigenic polynuclear aromatic hydrocarbons. To the best of our knowledge, a large amount of ergosterol is present in moldy cigarettes, which derives from fungal contaminations. Thus, the development of a simple, fast, and efficient method for the analysis of ergosterol is in great demand. In this paper, GC/MS following microwave-assisted silylation (MAS) was developed for the rapid quantitative analysis of ergosterol in cigarettes for the first time. In our work, total ergosterol in cigarettes after NaOH saponification was extracted with hexane, and then was fast derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) under microwave irradiation. Finally, the ergosterol trimethylsilyl derivative was analyzed by GC/MS. Derivatization conditions including microwave reaction solvent, irradiation time, and power were investigated. Method validations (linear range, LOD, precision, and recovery) were also studied. The results showed that the proposed method provided a fast, simple, and sensitive approach for the determination of ergosterol in cigarettes. Finally it was successfully applied to the analysis of ergosterol in normal and mildewy cigarettes

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Cancer Epidemiol Biomarkers Prev 2008 **17** (4) 945
Detection and quantitation of *N*'-nitrosonornicotine in human toenails by liquid chromatography-electrospray ionization-tandem mass spectrometry
The role of tobacco smoke exposure in human cancers requires specific biomarkers of tobacco carcinogen uptake. Two new biomarkers of human exposure to tobacco-specific carcinogens have been recently developed: urinary *N*'-nitrosonornicotine (NNN) and toenail 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). In this study, the presence of NNN in human toenails is reported. Toenails of 17 smokers were analyzed for total NNN. Mean total NNN level in these samples was 4.63 +/- 6.48 fmol/mg toenail and correlated with previously reported total NNAL (r = 0.96; P < 0.0001), total nicotine (r = 0.48; P < 0.05), and total cotinine (r = 0.87; P < 0.0001). It is noteworthy that amounts of NNN in smokers' toenails were generally higher than those of total NNAL. The ratio of toenail NNN to NNAL averaged 2.8, whereas the previously reported ratio between these biomarkers in smokers' urine was 0.1. NNN was also found in toenail samples from 12 nonsmokers, averaging 0.35 +/- 0.16 fmol/mg and positively correlating with toenail cotinine (r = 0.58; P = 0.05). The quantification of NNN in human toenails provides a potentially useful new biomarker of tobacco carcinogen exposure

15 Homeland Security

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Rapid Commun Mass Spectrom 2008 **22** (16) 2526
Microsynthesis and gas chromatography/electron ionization mass spectrometric analysis of cyclic alkylphonates for verification of the Chemical Weapons Convention
We describe the microsynthesis and gas chromatography/mass spectrometric (GC/MS) analysis of cyclic alkylphosphonates (CAPs), which are included in schedule 2B4 chemicals in the Chemical Weapons Convention (CWC). The reported microsynthesis is efficient in comparison with traditional synthesis. GC/MS and GC/tandem mass spectrometric (MS/MS) analysis of a variety of CAPs revealed that their fragmentations were dominated by α-cleavages, alkene eliminations and hydrogen rearrangements. Based on the obtained mass spectra and precursor and product ion analysis of five-, six- and seven-membered cyclic alkylphosphonates, the proposed fragmentation routes rationalize most of the characteristic ions

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J Chromatogr A 2008 **1196-1197** 125
Determination of basic degradation products of chemical warfare agents in water using hollow fibre-protected liquid-phase microextraction with in-situ derivatisation followed by gas chromatography-mass spectrometry
For the first time, the *in-situ* derivatisation and analysis of basic degradation products of chemical warfare agents in water samples was investigated by hollow fibre-protected liquid-phase microextraction (HF-LPME) together with gas chromatography-mass spectrometry. Nerve and blister agents, and a psychotomimetic agent degradation products were studied. A mixture of solvent and derivatising agent was successfully employed to extract with in-situ derivatisation. The hydrophobic hollow fibre was used to protect the moisture-sensitive derivatising agent. Parameters such as type of derivatising agent, extraction solvent, pH, salt concentration, stirring speed and extraction time were optimised using spiked deionised water samples. The linear range established was between 0.05 and 25 μg/ml depending on analyte, with squared regression coefficients ranging from 0.9959 to 0.9996. Relative standard deviations (RSDs) ranged from 6% to 10%. For comparison, solid-phase microextraction (SPME) was also evaluated and extraction conditions such as pH, salt concentration, stirring speed and extraction time were optimised. This work provides the first report of such an in-situ derivatisation approach for SPME of basic analytes. The linear range established was between 0.5 and 25 μg/ml depending on analyte, with squared regression coefficients ranging from 0.9946 to 0.9998. RSDs ranged from 5% to 22%. The limits of detection of HF-LPME (0.04-0.36 μg/l showed improvement over those of SPME (0.06-0.77 μg/l

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Rapid Commun Mass Spectrom 2008 **22** (12) 1971
Microsynthesis and mass spectral study of Chemical Weapons Convention related 2-alkyl-1,3,6,2-dioxathia-phosphocane-2-oxides
Not on Pub Med

Xu M, Terry AV, Bartlett MG*// *Univ Georgia, Coll Pharm, Dept Pharmaceut & Biomed Sci, Athens, Ga 30602, USA
Rapid Commun Mass Spectrom 2008 **22** (19) 3069
Determination of diisopropylfluorophosphate in rat plasma and brain tissue by headspace solid-phase microextraction gas chromatography/mass spectrometry
A simple, sensitive and rapid method for the determination of diisopropylfluorophosphate (DFP) in rat plasma and brain tissue using headspace solid-phase microextraction (HS-SPME) and gas chromatography/mass spectrometry (GC/MS) is presented. A 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was selected for sampling. The main parameters affecting the SPME process such as extraction and desorption temperature, extraction and desorption time, salt addition, and fiber preheating time were optimized in each matrix to enhance the extraction efficiency of the method. The lower limits of quantitation for DFP in plasma and brain tissue were 1 ng/ml and 3 ng/g, respectively. The method showed good linearity over the range from 1-100 ng/ml in plasma and 3-300 ng/g in brain tissue with correlation coefficient (r^2) values higher than 0.995. The precision and accuracy for intra-day and inter-day were less than 10%. The relative recoveries in plasma and brain for DFP were greater than 50%. Stability tests including autosampler and freeze and thaw were also investigated. This validated method was successfully applied to study the neurobehavioral effects of low-level organophosphate exposures

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Anal Chem 2008 **80** (17) 6671
Miniaturized low-cost ion mobility spectrometer for fast detection of chemical warfare agents
Hazardous compounds in air may be detected using ion mobility spectrometry (IMS). Regular applications are the detection of chemical warfare agents, highly toxic industrial compounds, explosives, and drugs of abuse. Detection limits in the low part per billion range, fast response times, and simple instrumentation make this technique more and more popular. Moreover, there is an increasing demand for miniaturized low-cost IMS for hand-held devices and air monitoring of public areas by sensor networks. Herein, we present a miniaturized aspiration condenser type ion mobility spectrometer for fast detection of chemical warfare agents. The device is easy to manufacture and provides single substance identification down to low part per billion-level concentrations within seconds. The improved separation performance results from ion focusing by means of geometric constraints and fluid dynamics. A simple pattern recognition algorithm is employed for the identification of trained

substances in air. The device was tested at the German Armed Forces Scientific Institute for Protection Technologies-NBC-Protection. Different chemical warfare agents, such as sarin, tabun, soman, US-VX, sulfur mustard, nitrogen mustard, and lewisite were tested. Results are included

16 Workplace

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Environ Sci Technol 2008 **42** (17) 6587

Permeation of tecnazene through human skin *in vitro* as assessed by HS-SPME and GC-MS

Human cadaver skin in vitro was utilized with CC-MS employing HS-SPME for receptor solution analyses of the permeation of tecnazene. Skin mounted on Franz diffusion cells and placed in a fume hood where two doses of tecnazene dissolved in acetone, corresponding to 103 and 864 μg/cm^2 of tecnazene, were applied. Cells were either occluded with aluminum foil or left unoccluded. Total absorption of tecnazene (dermis + receptor fluid) after 48 h was 2.2-6.1% of the applied dose for the unoccluded treatments and 22-33% for the occluded treatments. Potentially absorbed dose including all tecnazene that may have eventually permeated the skin ranged from 10% unoccluded to 42-53% occluded. Accumulation in the receptor solutions was satisfactorily described by a working diffusion model after upward adjustment of the partition coefficient for tecnazene in all skin layers by a factor of 5-16 *versus* a priori values. However, residual amounts of tecnazene in both the epidermis and dermis were higher than those estimated from the model, suggesting the prescence of tissue binding not accounted for in the calculation. The results suggest that the diffusion model as presently calibrated may significantly underestimate both systemic absorption and skin concentrations of highly lipophilic compounds, as predicted from data generated from in vitro skin permeation assays. Model predictions might be improved by more appropriate accounting of partitioning in the epidermis and dermis

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Fuel 2008 **87** (7) 1428

Characterization of asphalt fume composition by GC/MS and effect of temperature

A process at atmospheric pressure under air flow was developed in order to qualify and quantify volatile organic compounds (VOC) in asphalt emissions with a standard analytical protocol with gas chromatography coupled to mass spectrometry. Asphalt temperature, the asphalt oxidation, and the humidity of air determined VOC formation. VOCs were mainly correlated with the asphalt temperature

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Atmos Environ 2008 **42** (9) 2117

Use of REMPI-TOFMS for real-time measurement of trace aromatics during operation of aircraft ground equipment

Using a resonance enhanced multiphoton ionization–time of flight mass spectrometry (REMPI–TOFMS) system consisting of a pulsed solid state laser for photoionization and a TOFMS for mass discrimination, emissions of aromatic air toxics from aircraft ground equipment (AGE) were determined. This instrument provided characterization of turbine emissions and the effect of varying load operations on pollutant production. REMPI-TOFMS is capable of high selectivity and low detection limits (part per trillion to part per billion) in real time (1 s resolution). During startups and idle and full load operations, hazardous air pollutants and criteria pollutants were measured. Measurements of compounds such as benzene, toluene, ethylbenzene, xylenes, styrene, and polycyclic aromatic hydrocarbons compared well with standard methods. Startup emissions from the AGE data showed persistent levels of pollutants, unlike those from a diesel generator, where a sharp spike in emissions rapidly declined to steady state levels. The time-resolved responses of air toxics concentrations varied significantly with source, complicating efforts to minimize these emissions with common operating parameters. The time-resolved measurements showed that pollutant concentrations declined (up to 5 fold) in a species-specific manner over the course of multiple hours of operation, complicating determination of accurate and precise emission factors via standard extractive sampling. Correlations of air toxic concentrations with more routinely determined pollutants such as CO or PM were poor due to the relatively greater changes in the measured toxics' concentrations

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J Anal Toxicol 2008 **32** (4) 303

Determination of *p-tert*-octyl phenol in blood and tissues by gas chromatography coupled with mass spectrometry

p-tert-Octylphenol (OP), a persistent degradation product of alkylphenol ethoxylates that binds to the estrogen receptor in blood and tissues, has been analyzed with a sensitive and reproducible procedure using gas chromatography coupled with mass spectrometry. Blood (200 μl) or tissue homogenate (400 μl) was extracted with methyl tert-butyl ether, including *p-tert*-butylphenol (BP) as internal standard. Following extraction, the sample was evaporated to dryness with a gentle stream of nitrogen at 45°C, and OP and BP were derivatized with an acetylation reaction involving acetic anhydride and catalyzed by pyridine. A gas chromatograph equipped with a mass spectrometer (single ion monitoring) with a Varian VF-5ms capillary column was used in the analysis. The limits of detection and quantification of the method in blood were 4.6 and 15.5 ng/ml, respectively. The linearity and reproducibility of the method were acceptable, with coefficients of variation of approximately 10% for blood and ranging between 9% and 27% for tissues. Unchanged OP in blood and tissues obtained from Sprague-Dawley rats after oral and IV OP administration was analyzed by this method

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Anal Bioanal Chem 2008 **391** (6) 2249

Analysis of polybrominated diphenyl ethers (PBDEs) by liquid chromatography with negative-ion atmospheric pressure photoionization tandem mass spectrometry (LC/NI-APPI/MS/MS): Application to house dust

Using reverse-phase liquid chromatography on an octadecylsilane column, eight polybrominated diphenyl ether (PBDE) congeners of primary interest to the US EPA were separated. BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209 were baseline-resolved under isocratic conditions in 92:8 methanol/water (v/v). For the eight congeners studied, negative-ion atmospheric pressure photoionization (NI-APPI) with a toluene dopant produced precursor ions corresponding to $[M\text{-}Br+O]^-$. By employing tandem mass spectrometry with unique multiple reaction monitoring (MRM) transition, each congener was quantified . On-column limits of detection were between 2.4 and 27.8 pg for the eight congeners studied, with an intra-day method precision of 9%. The LC/NI-APPI/MS/MS method was validated for the analysis of the eight PBDE congeners in NIST SRM 2585 (Organics in House Dust). Pressurized liquid extraction (PLE) with subsequent LC/NI-APPI/MS/MS analysis afforded quantitative recovery for all eight PBDE congeners with recoveries ranging from 92.7 to 113%. The liquid-phase separation of the LC/NI-APPI/MS/MS technique was not subject to the thermal degradation issues that pervade splitless GC based analyses of highly brominated PBDEs such as BDE-209

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Rapid Commun Mass Spectrom 2008 **22** (16) 2573

Analysis of ethylenethiourea as a biomarker in human urine using liquid chromatography/triple quadrupole mass spectrometry

Ethylenebisdithiocarbamates (EBDCs) are widely used fungicides. Ethylenethiourea (ETU), the main metabolite and also a contaminant in the commercially available products, is of major toxicological concern. In this study, a method using liquid chromatography/triple quadrupole mass spectrometry (LC/MS/MS) is described for the analysis of ETU in human urine after a single-step extractive derivatization using pentafluorobenzyl bromide (PFBBr). Analysis was carried out using selected reaction monitoring (SRM) in the positive ion mode. Quantification of ETU was performed using [$^2H_4$]-labeled ETU as internal standard (IS). The limit of detection (LOD) was determined to 0.05 ng/ml. The method was linear in the range 0.1-54 ng/ml urine and had a within-run precision of 3-5%. The between-run precision was determined at an average urine level of 2 and 10 ng/ml urine and found to be 9%. The inter-batch precision was 6%. To validate ETU as a biomarker of exposure, the method was applied in a human experimental oral exposure to the commercial fungicide Ridomil Gold, containing 64% mancozeb and 4.5% ETU. Two healthy volunteers received 8.9 μg/kg body weight (b.w.) Ridomil Gold in a single oral dose followed by urine sampling for 104 h post-exposure. The elimination half-life of ETU was estimated to 17-23 h

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Atmos Environ 2008 **42** (8) 1801

Polycyclic aromatic hydrocarbons (PAHs) in indoor dust matter of Palermo (Italy) area: Extraction, GC-MS analysis, distribution and sources

Since people spend more than 80% of their time indoor environments, studies on indoor pollution are important. Herein, a technique for PAHs analysis in indoor dust (used as passive sampler) and the results relative to samples collected in the area of Palermo are developed. Dust samples for analysis were collected from 45 indoor environments. Total PAHs concentrations in indoor dusts ranged from 36 to 34 453 μg/kg d.w. To correlate indoor and outdoor pollution, the particulate matter and PAHs levels samples collected in four

stations were investigated. The percentage measured in indoor dusts was lower than that found outdoors. Isomeric ratios values for the different samples were used to identify the predominant PAHs sources

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J Environ Sci Health B 2008 **43** (4) 323

Trace level determination of selected organophosphorus pesticides and their degradation products in environmental air samples by liquid chromatography-positive ion electrospray tandem mass spectrometry

A new analytical method for determination of organophosphorus pesticides (OPs) together with their degradation products involving liquid chromatography (LC) positive ion electrospray (ESI^+) tandem mass spectrometry (MS-MS) with selective reaction monitoring (SRM) is decribed. Chromatography was performed on a Gemini C6-Phenyl (150 mm x 2.0 mm, 3 µm) with a gradient elution using water-methanol with 0.1% formic acid, 2 mM ammonium acetate mobile phase at a flow rate of 0.2 ml/min. The LC separation and MS/MS operating conditions were optimized with a total analysis time less than 40 minutes. Method detection limits of 0.1-5 µg/l for selected organophosphorus pesticides (OP), OP oxon degradation products, and other degradation products: 3,5,6-trichloro-2-pyridinol (TCP); 2-isopropyl-6-methyl-4-pyrimidol (IMP); and diethyl phosphate (DEP). Some OPs such as fenchlorphos are less sensitive (MDL 30 µg/l). Calibration curves were linear with coefficients of correlation better than 0.995. A three-point identification approach was adopted with area from first selective reaction monitoring (SRM) transition used for quantitative analysis, while a second SRM transition along with the ratio of areas obtained from the first to second transition are used for confirmation with sample tolerance established by the relative standard deviation of the ratio obtained from standards. By employing this new method, the first known detection of OP oxon degradation products including chlorpyrifos oxon at Bratt's Lake, SK and diazinon oxon and malathion oxon at Abbotsford, BC in atmospheric samples was achieved. Atmospheric detection limits typically ranged from 0.2-10 pg/m^3

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Rapid Commun Mass Spectrom 2008 **22** (17) 2629

Simultaneous determination of mercapturic acids derived from ethylene oxide (HEMA), propylene oxide (2-HPMA), acrolein (3-HPMA), acrylamide (AAMA) and *N,N*-dimethylformamide (AMCC) in human urine using liquid chromatography/tandem mass spectrometry

Mercapturic acids are highly important and specific biomarkers of exposure to carcinogenic substances in occupational and environmental medicine. We have developed and validated a reliable, specific and very sensitive method for the simultaneous determination of five mercapturic acids derived from several high-production chemicals used in industry, namely ethylene oxide, propylene oxide, acrylamide, acrolein and *N,N*-dimethylformamide. Analytes are enriched and cleaned up from urinary matrix by offline solid-phase extraction. The mercapturic acids are subsequently separated by means of high-performance liquid chromatography on a Luna C8 (2) column and specifically quantified by tandem mass spectrometric detection using isotopically labelled analytes as internal standards. The limits of detection (LODs) for *N*-acetyl-*S*-2-carbamoylethylcysteine (AAMA) and *N*-acetyl-*S*-2-hydroxyethylcysteine (HEMA) were 2.5 µg/l and 0.5 µg/l urine, while for *N*-acetyl-*S*- 3-hydroxypropylcysteine (3-HPMA), *N*-acetyl-*S*-2-hydroxypropylcysteine (2-HPMA) and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) it was 5 µg/l. These LODs were sufficient to detect the background exposure of the general population. We applied the method on spot urine samples of 28 subjects of the general population with no known occupational exposure to these substances. Median levels for AAMA, HEMA, 3-HPMA, 2-HPMA and AMCC in non-smokers (n = 14) were 52.6, 2.0, 155, 7.1 and 113.6 µg/l, respectively. In smokers (n = 14), median levels for AAMA, HEMA, 3-HPMA, 2-HPMA and AMCC were 243, 5.3, 1681, 41.7 and 822 µg/l, respectively. Due to the simultaneous quantification of these mercapturic acids, our method is well suited for the screening of workers with multiple chemical exposures as well as the determination of the background excretion of the general population

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J Anal Toxicol 2008 **32** (4) 273

Quantification of fuel oxygenate ethers in human blood using solid-phase microextraction coupled with gas chromatography-high-resolution mass spectrometry

Fuel oxygenates have a high water solubility and slow degradation rate. Their widespread use has resulted in an increase in the potential for human exposure. An accurate, precise, sensitive, and high-throughput analytical method to simultaneously quantify trace levels (low parts-per-trillion) of four fuel oxygenates in human blood: methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), di-isopropyl ether (DIPE), and *tert*-amyl methyl ether (TAME) is proposed. Analytes were extracted from the head space above human blood samples, using solid-phase microextraction, desorbed into the heated injector, and chromatographically resolved by capillary gas chromatography. They were analyzed by high-resolution mass spectrometry with multiple ion monitoring, and quantified against known standard levels by use of stable isotope-labeled internal standards for recovery correction. The low limits of detection (0.6 ng/l) allowed for measurement of MTBE, ETBE, DIPE, and TAME in parts-per-trillion levels with excellent precision (coefficient of variation ranging from 1.7 to 5.4%) and accuracy (96-100%). This technique provides a means to assess fuel oxygenate exposure and study the potential relationship between exposure and adverse health outcomes

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Talanta 2008 **76** (4) 865

Automated on-line column-switching HPLC-MS/MS method for measuring environmental phenols and parabens in serum

Serum concentrations of seven environmental phenols and five parabens: bisphenol A; *ortho*-phenylphenol; 2,4-dichlorophenol; 2,5-dichlorophenol; 2,4,5-trichlorophenol; benzophenone-3; triclosan; and methyl-, ethyl-, propyl-, butyl-, and benzyl-parabens were measured by development a method using on-line solid phase extraction (SPE) coupled to high performance liquid chromatography-isotope dilution tandem mass spectrometry (HPLC-MS/MS). The phenols and parabens present in serum were retained and concentrated on a C_{18} reversed-phase size-exclusion SPE column, back-eluted from the SPE column while the eluate was diluted through a mixing Tee (analyte peak focusing), separated using a pair of monolithic HPLC columns, and detected by isotope dilution-MS/MS. Sample preparation did not require protein precipitation, only dilution of the serum with 0.1M formic acid. This technique, which combined an on-line SPE with analyte peak focusing feature and the selective atmospheric pressure photoionization MS detection, resulted in limits of detection ranging from 0.1 to 0.5 ng/ml for most of the analytes. The high throughput and adequate sensitivity with yet a relative low serum volume used (100 µl) confirm that analytically it is possible to simultaneously determine these phenols and parabens with the precision and accuracy at sub-parts-per-billion levels required for biomonitoring. However, important additional factors, including validated sample collecting, handling, and storing protocols, as well as toxicokinetic data, are required if these measures are used for exposure assessment

17 Product Authenticity

Date H, Toyota A, Terauchi M, Sugimura M, Matsuo T, Mochiike C// Hiroshima Prefectural Technol Res Inst, Hlth & Environm Ctr, 1-6-29 Minami machi, Minami ku, Hiroshima 734 0007, Japan

Yakugaku Zasshi 2008 **128** (5) 811

Rapid determination of medical components found in the health food for weight loss by liquid chromatography/tandem mass spectrometry (LC/MS/MS) (Japanese, English Abstract)

The rapid determination of 11 medical components found in health foods for weight loss using liquid chromatography/tandem mass spectrometry (LC/MS/MS) has been developed. HPLC separation was carried out on an ODS column with the gradient elution method. The mobile phase consisted of two solvents. Solvent A was water and solvent B was methanol/acetonitrile (1:1), and both contain 0.1% formic acid and 5 mmol/l of ammonium acetate. Each medical component wass analyzed with multiple-reaction monitoring (MRM) in both negative and positive modes through electrospray ionization (ESI). The recovery rates of the 11 medical components added to commercially available health foods were 46.3-114% and each coefficient of variation was 13.7% or less. It was validated that this method may be applied to the urgent analysis of health foods that have caused damage to health

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J Chromatogr Sci 2008 **46** (8) 707

Simultaneous determination of sibutramine and *N*-di-desmethylsibutramine in dietary supplements for weight control by HPLC-ESI-MS

The simultaneous determination of the illegal additives sibutramine and its metabolite *N*-di-desmethylsibutramine in dietary supplements for weight control is reported by the development of a high-performance liquid chromatographic method, coupled with UV detection and electrospray ionization mass spectrometry (HPLC-UV-ESI-MS). Separation was achieved on a Spherisorb C8 reversed-phase column, employing acetonitrile and an aqueous 0.2% formic acid solution containing 20mM ammonium acetate as mobile phases in a gradient mode. UV detection was used for quantitation at a wavelength of 223 nm. Identification of target compounds is completed by ESI-MS using selected ion recording at *m/z* 280 for sibutramine and *m/z* 252 for *N*-di- desmethylsibutramine. Calibration curves were linear over the range of 0.025-1.0 mg/ml

for sibutramine and *N*-di-desmethylsibutramine. Correlation coefficients are better than 0.9990. The intra- and inter-day precision and accuracy for sibutramine and *N*-di-desmethylsibutramine were acceptable. The technique was successfully applied to the analysis of natural dietary supplement samples

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J Pharm Biomed Anal 2008 **47** (4-5) 786
Counterfeit homeopathic medicinal products: Syrups - A simple and rapid LC-ESI-MS method to detect preservatives not declared in label
Six preservatives in homeopathic syrups were simultaneously detected and quantified by a rapid and simple LC-ESI-MS technique. Counterfeit homeopathic syrups are suspected to contain preservatives that are not declared in label. Consequently, a method to ascertain the absence of sorbic and benzoic acids, methyl-, ethyl-, propyl- and butyl-parabens, as the most frequently utilised preservatives, was developed. Analytes were eluted with a linear gradient of acetonitrile-5mM ammonium acetate in 12 min using 2,4-dichlorobenzylalchol as Internal Standard. The HPLC separation was performed on an Eclipse XDB-C_{18} (2.1 mm x 50 mm-5 µm) column and the ESI-MS detection was performed in negative ion mode. Linearity of the method was studied in the range of 2 pg to 10 ng injected and correlation coefficients r^2 > or =0.9992 were obtained. LOD ranged from 0.04 to 0.4 ng/ml

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J Anal Atom Spectrom 2008 **23** (9) 1294
Detection of counterfeit tablets of an antiviral drug using ^{34}S measurements by MC-ICP-MS and confirmation by LA-MC-ICP-MS and HPLC-MC-ICP-MS
By means of laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICP-MS) and high performance liquid chromatography multicollector inductively coupled plasma mass spectrometry (HPLC-MC-ICP-MS) a new approach for pharmaceutical counterfeit detection has been developed. Helium has been used as the carrier gas for better sensitivity and laser ablation and chromatographic separation parameters have been previously optimised. A consistency study of 288 pharmaceutical tablets from different batches of the genuine drug has been performed by MC-ICP-MS and characteristic sulfur isotopic signature has been obtained for the genuine product. ^{34}S measurements by MC-ICP-MS using silicon internal standardisation for the correction of instrumental mass bias effects led to a ^{34}S of 3.6 and an associated combined expanded uncertainty of 1 (k = 2). A S-containing compound, the active pharmaceutical ingredient (API) of the tablets, was separated by HPLC and sulfur isotope amount ratios have been measured by MC-ICP-MS. ^{34}S values obtained by HPLC-MC-ICP-MS for the genuine tablets agreed with those obtained by laser ablation MC-ICP-MS, thereby confirming that the sulfur isotopic signature is inherent to the S-containing API. A blind exercise was performed for four hundred tablets after the initial development work. The discriminating power of the technique was assessed and uncertainties associated to ^{34}S values for counterfeit and genuine tablets varied depending on the sample introduction technique utilised. The three approaches were able to distinguish genuine from counterfeit tablets and four distinct groups of counterfeit tablets were detected. Therefore, the MC-ICP-MS method has potential as a rapid screening tool for pharmaceutical counterfeit detection and classification.

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Food Addit Contam 2008 **25** (7) 822
Analysis of six synthetic adulterants in herbal weight-reducing dietary supplements by LC electrospray ionization-MS
The simultaneous determination of six synthetic adulterants, namely fenfluramine, phenolphthalein, *N*-di-desmethyl sibutramine, *N*-mono-desmethyl sibutramine, sibutramine, and orlistat was achieved by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). Herbal weight-reducing dietary supplements were analyzed with this method. Chromatographic separation of the analytes on a C_8 reversed-phase column was achieved using a gradient elution of solvent A: acetonitrile and solvent B: aqueous 20 mM ammonium formate solution. Sildenafil was utilized as an internal standard for quantification. The MS detector was operated in positive electrospray ionization mode. Selected-ion monitoring (SIM) was carried out for *m/z* 232, 319, 252, 266, 280, 496, and 475 for fenfluramine, phenolphthalein, *N*-di-desmethyl sibutramine, *N*-mono-desmethyl sibutramine, sibutramine, orlistat, and sildenafil, respectively. The method was validated for accuracy, precision, linearity, and selectivity. The limits of detection for the six synthetic adulterants ranged from 0.0018 to 0.73 µg/g. The technique was emplyed for a small survey of 22 dietary supplements of which eleven samples were adulterated with phenolphthalein, *N*-mono-desmethyl sibutramine, and sibutramine at levels from 0.212 to 96.2 mg/g

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Food Chem 2009 **113** (1) 227
Simultaneous of illegal additives in dietary supplements and traditional medicines by high performance liquid chromatography-electrospray ionization mass spectrometry
The simultaneous determination of phenformin, rosiglitazone, glibenclamide and glimepiride in dietary supplements and traditional Chinese medicines for diabetes mellitus was investigated using a high performance liquid chromatographic method coupled with electrospray ionization mass spectrometry (HPLC/ESI-MS). Separation was achieved on a C_{18} column with the mobile phase consisted of acetonitrile and water (0.05% formic acid (v/v) and 20 mM ammonium acetate), at a flow rate of 1.0 ml/min with gradient elution. ESI-MS was employed to identify and quantify the analytes. Sildenafil was employed as internal standard. Full validation of the proposed method was provided (selectivity, linearity, limit of detection, limit of quantification, precision and accuracy). By contrast to previous methods, the proposed method has obvious advantages of simplicity, rapidity, accuracy and good applicability, and it has been applied to analyze illegal additives in nine dietary supplements and eight traditional Chinese medicines successfully

18 Techniques

Arraez-Roman D, Zurek G, Bassmann C, Segura-Carretero A*, Fernandez-Gutierrez A// *Univ Granada, Fac Sci, Dept Anal Chem, Res Grp FQM 297, C/ Fuentenueva s/n, ES-18071 Granada, Spain
Electrophoresis 2008 **29** (10) 2112
Characterization of *Atropa belladonna* L. compounds by capillary electrophoresis electrospray ionization-time of flight-mass spectrometry and capillary electrophoresis electrospray ionization-ion trap-mass spectrometry
This short communication describes the characterization of seven tropane alkaloid compounds in *Atropa belladonna* L. Thus a rapid and easy CE-electrospray interface (ESI)-TOF-MS procedure is developed to analyze these compounds in a pharmaceutical preparations of *A. belladonna* L. leaf extract. Optimum electrophoretic separation is obtained using an alkaline solution of 60 mM ammonium acetate at pH 8.5 containing 5% isopropanol. Under the optimum CE-ESI-TOF-MS conditions several important compounds such as tropine, belladonnine, norhyoscyamine, apoatropine, hyoscyamine, 6β-hydroxyhyoscyamine, and scopolamine have been simultaneously identified from *A. belladonna* L. CE-ESI-IT-MS has been used to discriminate the putative presence of littorine. The sensitivity, together with mass accuracy and true isotopic pattern of the TOF-MS, allowed the identification of a broad series of tropane alkaloid compounds present in pharmaceutical preparations of *A. belladonna* L. leaf extract

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Electrophoresis 2008 **29** (10) 2182
Determination of trace levels of benzodiazepine in urine using capillary electrochromatography-time of flight mass spectrometry
This paper details a method for the separation and determination of ten benzodiazepines in urine using CEC-MS(TOF) and an hexyl acrylate-based porous monolith. The TOF mass spectrometer provides an exact mass of protonated benzodiazepines to three decimal places (1-6 ppm). This high selectivity along with the CEC separation, provides an effective method for the identification of benzodiazepines. Linearity is satisfactory for all compounds in the concentration range of 25-500 ng/ml for lorazepam and 12.5-500 ng/ml for the others. The RSDs are between 1.4-2.3% for retention times and 1.1-9.2% for relative areas. Using the monolithic stationary phase, a preconcentration step is achievable and permits an 75-140-fold improvement in sensitivity. This strategy permits the quantification of these drugs down to 1 ng/ml in urine. This method was used for the analysis of benzodiazepines in spiked urine samples

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Anal Chem 2008 **80** (14) 5648
MALDI-FTICR imaging mass spectrometry of drugs and metabolites in tissue
Imaging mass spectrometry analysis of drugs and metabolites in tissue using matrix-assisted laser desorption ionization-Fourier transform ion cyclotron resonance (MALDI-FTICR) is described as a new approach to the subject. The method makes use of the high resolving power to produce images from thousands of ions measured during a single mass spectrometry (MS)-mode experiment. Accurate mass measurement allows molecular specificity for the ion images on the basis of elemental composition. Accurate mass fragment ions generated in an external quadrupole-collision cell are employed for structural confirmation of the targeted compound. The ability to image many small molecules in a single measurement with high specificity is a significant improvement over existing MS/MS based technologies. Example images are shown for olanzapine in kidney and liver and imatinib in glioma

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Ther Drug Monit 2008 **30** (4) 467

Quantification of citalopram or escitalopram and their demethylated metabolites in neonatal hair samples by liquid chromatography-tandem mass spectrometry

Selective serotonin reuptake inhibitors such as citalopram and escitalopram are widely used in the treatment of depression. Adverse drug reactions and side effects have been reported. The development of specific methods for their determination is of great interest in both clinical and forensic toxicology. A liquid chromatography-tandem mass spectrometry method has been developed and validated for the assay of citalopram, escitalopram, and their demethylated metabolites in 10-mg hair samples. The analytes were extracted by incubation in methanol and liquid/liquid extraction with diethyl ether/dichloromethane. Gradient elution on a narrow bore C_{18} column was realized using clomipramine-d_3 as an internal standard. Positive ion electrospray ionization and tandem mass spectrometry determination by collision-induced dissociation were performed in an ion trap mass spectrometer. The method exhibited a linear range of 25 to 2000 pg/mg, a quantification limit of 25 pg/mg for all analytes, relative standard deviations in the range of 12.10 to 9.80 (intraassay), and 13.80 to 11.78 (interassay), and accuracies (as percent recovery of the spiked standards) in the range of 90% to 110%; it was applied to the determination of citalopram and escitalopram and their metabolites in hair samples of two newborns to document their *in utero* exposure to the drugs. The technique proved suitable for neonatal hair investigation for citalopram or escitalopram and was applied to two real cases of gestational exposure

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Anal Chem 2008 **80** (8) 2744

Easy ambient sonic-spray ionization mass spectrometry combined with thin-layer chromatography

On-spot detection and analyte characterization on thin-layer chromatography (TLC) plates was performed using ambient desorption/ionization and (tandem) mass spectrometry detection, which is *via* easy ambient sonic spray ionization mass spectrometry (EASI-MS). For confirmation, cases, mixtures of semipolar nitrogenated compounds as well as pharmaceutical drugs and vegetable oils have been tested. The technique was also employed to monitor a chemical reaction of synthetic importance. EASI is the simplest and gentlest ambient ionization technique currently available, assisted solely by N_2 (or air). It employs no voltages, no electrical discharges; no UV or laser beams, and no high temperature and is most easily implemented in all API mass spectrometers. In addition, TLC is the simplest, fastest, and most easily performed chromatographic technique. Therefore, TLC plus EASI-MS provides a simple and advantageous combination of chromatographic separation and sensitive detection of the TLC spots as well as on-spot MS or MS/MS characterization. The favorable characteristics of TLC-EASI-MS indicate suitability for applications in several areas such as drug and oil analysis, phytochemistry and synthetic chemistry, forensics *via* reliable counterfeit detection, and quality control

Le Breton MH, Rochereau-Roulet S, Pinel G, Bailly-Chouriberry L, Rychen G, Jurjanz S, Goldman T, Le Bizec B// Ecole Nat Veterinaire Nantes, Lab Etude Residus & Contaminants Aliments, BP 50707, FR-44307 Nantes, France

Rapid Commun Mass Spectrom 2008 **22** (20) 3130

Direct determination of recombinant bovine somatotropin in plasma from a treated goat by liquid chromatography/high-resolution mass spectrometry

Recombinant bovine somatotropin (rbST) is used in dairy cattle to enhance milk production. Despite the ban on this hormone in some countries, especially in Europe, there is so far no method available for the direct detection of rbST either in milk or in plasma. An analytical strategy has been developed to analyze rbST in plasma, including a purification procedure based on a precipitation with ammonium sulphate, followed by a solid-phase extraction (SPE)-based clean-up on C_4 sorbent and precipitation with cold methanol. The hormone was then digested with trypsin and analyzed by liquid chromatography/high-resolution mass spectrometry (LC/HRMS^n) on a linear ion trap coupled with an Orbitrap. The tryptic N-terminal peptide, specific to the difference between the endogenous and recombinant form of the somatotropin, was fragmented and product ions were analyzed at high mass resolution. Applying this approach to goat plasma allowed the direct detection of 10 ng/ml of rbST in fortified samples. It also showed the presence of rbST in plasma collected from a goat treated with the hormone, even 2 days after administration. These results are of a great interest in the field of somatotropin control and undoubtedly constitute a first step in the development of a method for the detection of rbST not only in bovine plasma, but also in other biological matrices such as milk

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J Anal Toxicol 2008 **32** (5) 355

A new method for simultaneous determination of cyclic antidepressants and their metabolites in urine using enzymatic hydrolysis and fast GC-MS

Six commonly prescribed cyclic antidepressants and their major metabolites in urine were simultaneously determined. The technique can be used for quantitation of amitriptyline, nortriptyline, imipramine, desipramine, doxepin, desmethyldoxepin, and maprotiline in human urine, in addition to the qualitative determination of their hydroxylated metabolites. It is suitable for confirmation of drug abuse in health care professionals and overdose cases where the identity of the abused cyclic antidepressant may not be known. Samples are spiked with internal standard and hydrolyzed with β-glucuronidase from *Escherichia coli*. Since the tertiary cyclic antidepressants were found to be extensively conjugated in urine, hydrolysis was found to be essential to the extraction procedure. In contrast, the secondary cyclic antidepressants were found to be minimally conjugated. Drugs are extracted from alkalinized urine into solvent and derivatized with MSTFA/ammonium iodide/ethanethiol reagent. This reagent produces more stable derivatives compared to reagents previously employed. Gas chromatographic (GC)-mass spectrometric analysis was performed in electron ionization mode by selective ion monitoring, using hydrogen as a carrier gas, a short narrow bore GC capillary column, and fast temperature program, allowing for a rapid analytical cycle. While maintaining specificity for these drugs, concentrations in human urine ranging from 50 to 20,000 ng/ml may be measured with intraday and interday precisions, expressed as variation coefficient, of less than 2.8% for all analytes

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Rapid Commun Mass Spectrom 2008 **22** (21) 3427

Simultaneous analysis of thirteen diuretics residues in bovine milk by ultra-performance liquid chromatography/tandem mass spectrometry

A simple, rapid, sensitive and specific method used to screen and confirm multi-class diuretics residues in whole bovine milk is described. Thirteen drugs of four different classes including carbonic anhydrase inhibitors, loop, thiazide and potassium-sparing diuretics were extracted from whole milk by acetonitrile followed by further purification with hexane. The analytes were separated using an ACQUITY UPLC BEH C_{18} column and detected by electrospray ionization tandem mass spectrometry (ESI-MS/MS). MS data acquisition was performed by a time-scheduled multiple reaction monitoring program, selecting two ion transitions for each target compound. The overall average recoveries based on matrix-fortified curves fortified with diuretics at three levels ranged from 80.6 to 108.8% with the coefficients of variation ranging from 2.6 to 19.7% (n = 6). The limits of quantitation (LOQs) of diuretics in bovine milk were 5.0 μg/kg for spironolactone and 0.5 μg/kg for other analytes, respectively

Thienpont LM, Van Uytfanghe K, Blincko S, Ramsay CS, Xie H, Doss RC, Keevil BG, Owen LJ, Rockwood AL, Kushnir MM, Chun KY, Chandler DW, Field HP, Sluss PM// Univ Ghent, Fac Pharmaceut Sci, Anal Chem Lab, Harelbekestr 72, BE-9000 Ghent, Belgium

Clin Chem 2008 **54** (8) 1290

State-of-the-art of serum testosterone measurement by isotope dilution-liquid chromatography-tandem mass spectrometry

The lack of accuracy of direct immunoassays has resulted in the development of serum testosterone assays based on isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS). The accuracy and state of standardization (traceability) of 4 published ID-LC-MS/MS procedures in a method comparison with an ID-gas chromatography (GC)-MS reference measurement procedure listed in the database of the Joint Committee for Traceability in Laboratory Medicine was compared. The study used 58 specimens from different patient categories. Each specimen was measured in triplicate (ID-LC-MS/MS) and quadruplicate (ID-GC-MS) in independent runs. The testosterone concentrations by ID-GC-MS were 0.2-4.4 nmol/l (women), 0.2-2.0 nmol/l (hypogonadal man), and 10.1-31.3 nmol/l (normogonadal men). For ID-GC-MS, the CV was nearly constant, with a median of 1.0%; for ID-LC-MS/MS, it was concentration-dependent, with a median of up to 8%. Weighted Deming regression gave mean slopes, intercepts, and correlation coefficients of 0.90-1.11, -0.055-0.013 nmol/l, and 0.993-0.997, respectively. The % difference plot showed between 7% and 26% of the results outside a total error limit of 14%, with median deviations from ID-GC-MS between -9.6 and 0.4%. This study demonstrated fairly good accuracy and standardization of the tested ID-LC-MS/MS procedures. Performance differences between procedures were evident in some cases due to improper calibration and between-run calibration control. This emphasizes the need for thorough validation, including traceability, of new ID-LC-MS/MS procedures