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Current awareness in drug testing and analysis

1 Reviews

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J Forensic Sci 2010 **55** (2) 451
Paradigm shift for the alcohol breath test
The alcohol breath test (ABT) has been used for quantification of ethyl alcohol in individuals suspected of driving under the influence for more than 50 years. In this time, there has been little change in the concepts underlying this single breath test. The old model, which assumes that end-exhaled breath alcohol concentration is closely related to alveolar air alcohol concentration, is no longer acceptable. This paper reviews experimental research and mathematical modeling which has evaluated the pulmonary exchange processes for ethyl alcohol. Studies have shown that alcohol exchanges dynamically with the airway tissue both during inspiration and expiration. The airway tissue interaction makes it impossible to deliver air with alveolar alcohol concentration to the mouth. It is concluded that the ABT is dependent on physiological factors that need to be assessed for accurate testing

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Int J Clin Pract 2010 **64** (4) 497
Counterfeit phosphodiesterase type 5 inhibitors pose significant safety risks
Counterfeit drugs are inherently dangerous and a growing problem; counterfeiters are becoming increasingly sophisticated. Growth of the counterfeit medication market is attributable in part to phosphodiesterase type 5 inhibitor (PDE5i) medications for erectile dysfunction (ED). Millions of counterfeit PDE5is are seized yearly and account for the bulk of all counterfeit pharmaceutical product seizures. It has been estimated that up to 2.5 million men in Europe are exposed to illicit sildenafil, suggesting that there may be as many illegal as legal users of sildenafil. Analysis of the contents of counterfeit PDE5is shows inconsistent doses of active pharmaceutical ingredients (from 0% to > 200% of labelled dose), contaminants (including talcum powder, commercial paint and printer ink) and alternative ingredients that are potentially hazardous. In one analysis, only 10.1% of samples were within 10% of the labelled tablet strength. Estimates place the proportion of counterfeit medications sold over the Internet from 44% to 90%. Of men who purchase prescription-only medication for ED without a prescription, 67% do so using the Internet. Counterfeit PDE5is pose direct and indirect risks to health, including circumvention of the healthcare system. More than 30% of men reported no healthcare interaction when purchasing ED medications. Because > 65% actually had ED, these men missed an opportunity for evaluation of comorbidities (e.g. diabetes and hypertension). Globally, increased obstacles for counterfeiters are necessary to combat pharmaceutical counterfeiting, including fines and penalties. The worldwide nature of the counterfeit problem requires proper coordination between countries to ensure adequate enforcement. Locally, physicians who treat ED need to inform patients of the dangers of ordering PDE5is *via* the Internet

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Intern Med J 2010 **40** (3) 183
Post-mortem drug concentrations
Considerable changes occur in the concentrations of numerous drugs after death. Some concentrations increase, others fall and some do not change. Active agents, such as γ hydroxybutyrate and alcohol, may be synthesized in the deceased body. Many published tables contain lists of therapeutic, toxic and lethal concentrations that are misleading. While the process of post-mortem redistribution of drugs has been called a 'toxicological nightmare', it is still possible to obtain a lot of information from the integration of ante-mortem history with the post-mortem drug concentration

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Ther Drug Monit 2010 **32** (1) 11
Poppy seed foods and opiate drug testing—where are we today?
Seeds of the opium poppy are legally available and routinely consumed in food. However, the seeds may contain morphine and other opiate alkaloids because of contamination during harvesting. This paper reviews the effects of poppy seed foods on opiate drug tests. Computer-assisted searching revealed 95 relevant papers. Poppy seed consumption is normally regarded as safe and during food processing, the morphine content is greatly reduced (up to 90%). However, the possibility of false-positive opiate drug tests following poppy food ingestion exists. Unfortunately, there are no unambiguous biomarkers which differentiate between poppy food ingestion and heroin or pharmaceutical morphine use. This is also presents a problem for heroin-assisted maintenance programs where a basic necessity is the patients' abstinence from any other drugs, including additional illicit heroin. Furthermore, a dearth of forensic ingestion trials was located that consider all factors influencing the morphine content in biologic matrices after consumption. Most studies did not control for the losses during food processing and thus the initial morphine dosage was overestimated. The large reduction of the morphine content during past years

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.

raises questions about the validity of the "poppy seed defence." Currently, a threshold of poppy seed food consumption that would not lead to positive drug tests with certainty is not available. Therefore, investigations are necessary to examine whether the morphine content of today's foods still pose the possibility of influencing drug tests. In addition, future trials should take processing-related morphine losses into account

2 Sports Doping - General

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Accredit Qual Assur 2010 **15** (2) 133
The importance of cooling of urine samples for doping analysis
The authors comment on the absence of cooled transport of samples for urinalysis. Storage and transportation of samples of urine for doping analysis, as performed by the anti-doping organizations associated with the World Anti-Doping Agency, does not provide a specific procedure for cooled transport from the place of sampling to the analytical laboratory. However, low cost cooling facilities might easily be made available. Consequently, microbial and thermal degradation of compounds in the urine may occur. This might produce false negative or false positive results in subsequent doping analysis. This is condemned as a scientifically and morally unacceptable practice which is still allowed despite publications proving that immediate cooling is absolutely necessary. Considering the enormous societal consequences of positive tests, it is suggested that the absence of a controllable secure system during transport should be prohibited. This paper suggests a simple protocol, based on immediate cooling and cooled transport, which might easily be implemented in developed countries and at low cost.

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Rapid Commun Mass Spectrom 2010 **24** (8) 1124
Comprehensive plasma-screening for known and unknown substances in doping controls
Occasionally, doping analysis has been recognized as a competitive challenge between cheating sportsmen and the analytical capabilities of testing laboratories. Both have made immense progress during the last decades, but obviously the athletes have the questionable benefit of frequently being able to switch to new, unknown and untested compounds to enhance their performance. Thus, as analytical counteraction and for effective drug testing, a complementary approach to classical targeted methods is required in order to implement a comprehensive screening procedure for known and unknown xenobiotics. The present study provides a new analytical strategy to circumvent the targeted character of classical doping controls without losing the required sensitivity and specificity. Using 50 µl of plasma only, the method potentially identifies illicit drugs in low ng/ml concentrations. Plasma provides the biological fluid with the circulating, unmodified xenobiotics; thus the identification of unknown compounds is facilitated. After a simple protein precipitation, liquid chromatographic separation and subsequent detection by means of high resolution/high accuracy orbitrap mass spectrometry, the procedure enables the determination of numerous compounds from different classes prohibited by the World Anti-Doping Agency (WADA). A new hyphenated mass spectrometry technology was employed without precursor ion selection for higher collision energy dissociation (HCD) fragmentation experiments. Thus the mass spectra contained all the desired information to identify unknown substances retrospectively. The method was validated for 32 selected model compounds for qualitative purposes considering the parameters specificity, selectivity, limit of detection (< 0.1- 10 ng/ml), precision (9-28%), robustness, linearity, ion suppression and recovery (80-112%). In addition to the identification of unknown compounds, the plasma samples were simultaneously screened for known prohibited targets

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Rapid Commun Mass Spectrom 2010 **24** (11) 1595
Preventive doping control screening analysis of prohibited substances in human urine using rapid-resolution liquid chromatography/high-resolution time-of-flight mass spectrometry
Unification of the screening protocols for a wide range of doping agents has become an important issue for doping control laboratories. This study presents the development and validation of a generic liquid chromatography/ time-of-flight mass spectrometry (LC/TOFMS) screening method of 241 small molecule analytes from various categories of prohibited substances (stimulants, narcotics, diuretics, β_2-agonists, β-blockers, hormone antagonists and modulators, glucocorticosteroids and anabolic agents). It is based on a single-step liquid-liquid extraction of hydrolyzed urine and the use of a rapid-resolution liquid chromatography/high-resolution time-of-flight mass spectrometric system acquiring continuous full scan data. Electrospray ionization in the positive mode was used. Validation parameters consisted of identification capability, limit of detection, specificity, ion suppression, extraction recovery, repeatability and mass accuracy. Detection criteria were established on the basis of retention time reproducibility and mass accuracy. The suitability of the methodology for doping control was demonstrated with positive urine samples. The preventive role of the method was proved by the case where full scan acquisition with accurate mass measurement allowed the retrospective reprocessing of acquired data from past doping control samples for the detection of a designer drug, the stimulant 4-methyl-2-hexanamine, which resulted in re-reporting a number of stored samples as positives for this particular substance, when, initially, they had been reported as negatives

3 Steroids

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Rapid Commun Mass Spectrom 2010 **24** (7) 958
Feasibility of capillary liquid chromatography/microchip atmospheric pressure photoionization mass spectrometry in analyzing anabolic steroids in urine samples
We examined the feasibility of capillary liquid chromatography/microchip atmospheric pressure photoionization tandem mass spectrometry (capLC/microAPPI-MS/MS) for the analysis of anabolic steroids in human urine. The urine samples were pretreated by enzymatic hydrolysis (with β-glucuronidase from *Helix pomatia*), and the compounds were liquid-liquid extracted with diethyl ether. After separation the compounds were vaporized by microchip APPI, photoionized by a 10 eV krypton discharge lamp, and detected by selected reaction monitoring. The capLC/microAPPI-MS/MS method showed good sensitivity with detection limits at the level of 1.0 ng/ml, good linearity with correlation coefficients between 0.9954 and 0.9990, and good repeatability with relative standard deviations below 10%. These results demonstrate that microchip APPI combined with capLC/MS/MS provides a new potential method for analyzing non-polar and neutral compounds in biological samples

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Rapid Commun Mass Spectrom 2010 **24** (6) 749
Mass spectrometric characterization of tamoxifene metabolites in human urine utilizing different scan parameters on liquid chromatography/tandem mass spectrometry
Different liquid chromatographic/tandem mass spectrometric (LC/MS/MS) scanning techniques were considered for the characterization of tamoxifene metabolites in human urine for anti-doping purpose. Five different LC/MS/MS scanning methods based on precursor ion scan (precursor ion scan of *m/z* 166, 152 and 129) and neutral loss scan (neutral loss of 72 Da and 58 Da) in positive ion mode were assessed to recognize common ions or common losses of tamoxifene metabolites. The applicability of these methods was checked first by infusion and then by the injection of solution of a mixture of reference standards of four tamoxifene metabolites available in our laboratory. The data obtained by the analyses of the mixture of the reference standards showed that the five methods used exhibited satisfactory results for all tamoxifene metabolites considered at a concentration level of 100 ng/ml, whereas the analysis of blank urine samples spiked with the same tamoxifene metabolites at the same concentration showed that the neutral loss scan of 58 Da lacked sufficient specificity and sensitivity. The limit of detection in urine of the compounds studied was in the concentration range 10-100 ng/ml, depending on the compound structure and on the selected product ion. The suitability of these approaches was checked by the analysis of urine samples collected after the administration of a single dose of 20 mg of tamoxifene. Six metabolites were detected: 4-hydroxytamoxifene, 3,4-dihydroxytamoxifene, 3-hydroxy-4-methoxytamoxifene, *N*-demethyl-4-hydroxytamoxifene, tamoxifene-*N*-oxide and *N*-demethyl-3-hydroxy-4-methoxytamoxifene, which is in conformity to our previous work using a time-of-flight (TOF) mass spectrometer in full scan acquisition mode

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Rapid Commun Mass Spectrom 2010 **24** (11) 1583

A sensitive and specific precursor ion scanning approach in liquid chromatography/electrospray ionization tandem mass spectrometry to detect methylprednisolone acetate and its metabolites in rat urine

A new, simple, sensitive and specific liquid chromatography/electrospray ionization tandem mass spectrometric (LC/ESI-MS/MS) method in precursor ion scanning (PIS) mode has been developed for the rapid detection of methylprednisolone acetate (MPA) and its metabolites in rat urine. A suitable product ion specific for methylprednisolone (MP) and MPA was selected after a fragmentation study on 20 (cortico)steroids at different collision energies (5-40 eV). Urine samples were simply treated with acetonitrile then dried in a SpeedVac system. The method was validated and compared with other PIS methods for detecting corticosteroids in human urine. It was more sensitive, with limit of detection (LOD) and lower limit of quantitation (LLOQ), respectively, of 5 and 10 ng/ml. The method was applied for the analysis of rat urine collected before and after (24, 48, 72 h) intra-articular (IA) injection of a marketed formulation of MPA (Depo-Medrol®). MS/MS acquisitions were taken at different collision energies for the precursor ions of interest, detected in PIS mode, to verify the MP-related structure. Six different metabolites were detected in rat urine, and their chemical structures were assigned with a computational study

4 Peptides

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J Pharm Biomed Anal 2010 **51** (3) 633

Validation of a method for the quantitation of ghrelin and unacylated ghrelin by HPLC

The separation and quantitation of human acylated and unacylated (or des-acyl) ghrelin from aqueous solutions was achieved with an HPLC/UV procedure. This technique was validated with an original approach using accuracy profiles based on tolerance intervals for the total error measurement. The concentration range which exhibited good accuracy extended from 1.85 to 59.30 µM for acylated and 1.93 to 61.60 µM for unacylated ghrelin. Optimal temperature, pH and buffer for sample storage were determined. Unacylated ghrelin was discovered to be stable in all conditions tested. At 37 °C acylated ghrelin was stable at pH 4 but unstable at pH 7.4. The principal degradation product was unacylated ghrelin. The validated HPLC/UV procedure was employed to evaluate the binding of acylated and unacylated ghrelin to liposomes

8 Recreational Drugs - General

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Ther Drug Monit 2010 **32** (1) 40

Monitoring pregnant women's illicit opiate and cocaine use with sweat testing

Adverse maternal, fetal, and neonatal consequences associated with dependence on illicit drugs during pregnancy are a major public health concern. Pregnant volunteers (n = 39) who provided written informed consent for this Institutional Review Board-approved protocol provided sweat patches (n = 389) which were replaced approximately weekly, from study entry until delivery. Solid phase extraction and gas chromatography mass spectrometry was employed to analyze the patches for opiates (heroin, 6-acetylmorphine, 6-acetylcodeine, morphine and codeine) and cocaine (cocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester). Seventy-one percent (276) of collected sweat patches contained > or = 5 ng (limit of quantification) of one or more analytes. In 254 (65.3%), cocaine was discovered in concentrations ranging from 5.2 to 11,835 ng per patch with 154 of these high enough to satisfy the proposed Substance Abuse and Mental Health Services Administration guidelines for a confirmatory drug test (25 ng per patch). 6-Acetylmorphine was the principal opiate analyte documented in 134 patches (34.4%) with 11.3% exceeding the proposed opiate Substance Abuse and Mental Health Services Administration cut-off (25 ng per patch). Heroin was identified in fewer patches (77) but in a similar concentration range (5.3-345.4 ng per patch). Polydrug use was manifest by the presence of both cocaine and opiate metabolites in 136 (35.0%) patches. Sweat patch analysis is an effective approach for monitoring abstinence or illicit drug use relapse in this high-risk population of pregnant opiate- and/or cocaine-dependent women

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Forensic Sci Int 2010 **195** (1-3) 153

Can the prevalence of high blood drug concentrations in a population be estimated by analysing oral fluid? A study of tetrahydrocannabinol and amphetamine

Research has been untaken to estimate the prevalence of high blood concentrations of tetrahydrocannabinol and amphetamine in a population of drug users by analysing oral fluid (saliva). Five procedures were compared, including simple calculation procedures dividing the drug concentrations in oral fluid by average or median oral fluid/blood (OF/B) drug concentration ratios or linear regression coefficients, and more complex Monte Carlo simulations. Populations of 311 cannabis users and 197 amphetamine users from the Rosita-2 Project were investigated. Results indicate that Monte Carlo simulations might provide superior accuracies than simple calculations if good data on OF/B ratios are available. When using only 20 randomly selected OF/B ratios, a Monte Carlo simulation produced the best accuracy but not the best precision. Dividing by the OF/B regression coefficient produced acceptable accuracy and precision, and was, as such, the best technique. No technique provided acceptable accuracy if the prevalence of high blood drug concentrations was less than 15%. Dividing the drug concentration in oral fluid by the OF/B regression coefficient resulted in an acceptable estimation of high blood drug concentrations in a population. Therefore, it might provide valuable additional information on possible drug impairment, e.g. in roadside surveys of drugs and driving. If good data on the distribution of OF/B ratios are available, a Monte Carlo simulation may provide better accuracy

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Forensic Sci Int 2010 **194** (1-3) 108

Determination of amphetamine-type stimulants, ketamine and metabolites in fingernails by gas chromatography-mass spectrometry

The simultaneous qualification and quantification of methamphetamine (MA), amphetamine (AP), 3,4-methylenedioxy-*N*-methylamphetamine (MDMA), 3,4-methylenedioxy-*N*-amphetamine (MDA), ketamine (KET) and norketamine (NKT) in fingernails was achieved following the development and validation of a gas chromatography-mass spectrometry (GC-MS) technique. Fingernail samples (20mg) were washed with distilled water and methanol, digested with 1.0M sodium hydroxide at 95 °C for 30 min, and then extracted with ethyl acetate. Extract solutions were evaporated to dryness, derivatized using heptafluorobutyric anhydride (HFBA) at 60 °C for 30 min, and analyzed by GC-MS. The linear ranges were 0.1-20.0 ng/mg for AP, MDMA and NKT, 0.2-20.0 ng/mg for MA and MDA, and 0.4-20.0 ng/mg for KET, with the coefficients of determination (r^2 >or= 0.9989). Intra- and inter-day precisions were within 7.1% and 10.6%, respectively. Intra- and inter-day accuracies were -10.9% to 0.8% and -4.3% to 4.5%, respectively. Limits of detections (LODs) and the limits of quantifications (LOQs) for each analyte were lower than 0.094 ng/mg and 0.314 ng/mg, respectively. Recoveries were in the range of 72.3-94.9%. Average fingernail growth rates of two subjects for three years and six subjects for two months were 3.12 mm/month and 3.16 mm/month, respectively. In addition, the procedure proved to be suitable for the simultaneous detection and quantification of MA, MDMA, KET and their metabolites in fingernails

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Forensic Sci Int 2010 **195** (1-3) 160

Forensic analysis of hallucinogenic mushrooms and khat (*Catha edulis* Forsk) using cation-exchange liquid chromatography

In many countries, allucinogenic mushrooms (e.g. *Psilocybe* and *Panaeolus* species) as well as leaves and young shoots of the khat tree (*Catha edulis* Forsk) are prohibited drugs. For legal purposes, the exact concentration of the hallucinogenic alkaloids psilocin and psilocybin in mushrooms and the sympathomimetic alkaloids cathinone and cathine in khat are necessary. As a consequence of an increasing number of mushroom and khat seizures by German customs authorities, a convenient, comprehensive, quantitative technique is essential. An HPLC method based on cation-exchange liquid chromatography for

these rather "exotic" drugs has been developed which circumvents time-consuming multi-step sample preparation or chemical derivatization procedures. Following application of this technique, a number of different hallucinogenic fungi species and products that are generally distributed via the internet have been analysed [dried and fresh *Psilocybe cubensis* Singer as well as *P. cubensis* collected from "grow boxes", *Panaeolus cyanescens* Berkeley and Broome and so-called "philosopher stones" (sclerotia of *Psilocybe* species)]. Highest total amounts of psilocin were discovered in dried *P. cyanescens* with up to 3.00+/-0.24 mg per 100 mg. In addition, the distribution of khat alkaloids in different parts of the khat shoots has been examined. High concentrations of cathinone were not only been discovered in leaves but also in green parts and barks of stalks. Sample treatments for fresh mushroom and khat samples were analysed and optimised. Highest amounts of alkaloids were noted when fresh material was freeze-dried

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J Anal Toxicol 2010 **34** (1) 17

Simultaneous liquid chromatography-mass spectrometry quantification of urinary opiates, cocaine, and metabolites in opiate-dependent pregnant women in methadone-maintenance treatment

In order to monitor possible drug relapse in 15 pregnant heroin-dependent women, urine was collected three times/week from 284 patients and analysed for opiates, cocaine, and metabolites were quantified by liquid chromatography-mass spectrometry (LC-MS). Opiates were identified in 149 specimens (52%) with limits of quantification (LOQ) of 10-50 µg/l. Morphine, morphine-3-glucuronide, and/or morphine-6-glucuronide were positive in 121 specimens; 6-acetylmorphine, a biomarker of heroin ingestion, could be quantified in only 7. Heroin, 6-acetylcodeine, papaverine, or noscapine were not detected. From all 15 participants, 165 specimens (58%) were positive for one or more cocaine analytes (LOQ 10-100 µg/l). Ecgonine methylester (EME) and/or benzoylecgonine were the primary biomarkers of cocaine in 142. Anhydroecgonine methylester, a biomarker of smoked cocaine, was positive in six; cocaethylene and/or ecgonine ethylester, biomarkers of cocaine and ethanol co-ingestion, were identified in 25. Sixteen opiate- and 29 cocaine-positive specimens were identified at the current Substance Abuse Mental Health Services Administration cutoffs for total morphine (2000 µg/l), codeine (2000 µg/l), 6-acetylmorphine (10 µg/l), and benzoylecgonine (100 µg/l). Including 100 µg/l EME as a further urinary cocaine biomarker would identify 51 more positive specimens. Disparate distributions of opiate and cocaine biomarkers were noted with LC-MS as compared to gas chromatography-mass spectrometry analysis

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Rapid Commun Mass Spectrom 2010 **24** (10) 1475

A fast gas chromatography/mass spectrometry method for the determination of stimulants and narcotics in urine

A fast method has been developed for the simultaneous determination of 52 stimulants and narcotics excreted unconjugated in urine by gas chromatography/mass spectrometry (GC/MS). The procedure involves the liquid/liquid extraction of the analytes from urine at strong alkaline pH and the injection of the extract into a GC/MS instrument with a fast GC column (10 m x 0.18 mm i.d.); the short column allows the complete separation of the 52 analytes in a chromatographic run of 8 min. The method has been fully validated giving lower limits of detection (LLODs) satisfactory for its application to antidoping analysis as well as to forensic toxicology. The repeatability of the concentrations and the retention times are good both for intra- and for inter-day experiments (%CV of concentrations always lower than 15 and %CV of retention times lower than 0.6). In addition, the analytical bias is satisfactory (A% always >15%). The method proposed here would be particularly useful whenever there are time constraints and the analyses have to be completed in the shortest possible time

9 Stimulants

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J Chromatogr B 2010 **878** (1) 21

Development and validation of a high-throughput method for the quantitative analysis of D-amphetamine in rat blood using liquid chromatography/MS^3 on a hybrid triple quadrupole-linear ion trap mass spectrometer and its application to a pharmacokinetic study

Compounds exhibiting strong central nervous system stimulant effects include sympathomimetic drugs such as amphetamines. The parent drug in this class to which all others are structurally related is D-amphetamine [(+)-α-] methylphenetylamine. D-amphetamine is widely employed for the exploration of novel mechanisms involving the catecholaminergic system and for the validation of new behavioural animal models. A specific and high-throughput technique with minimal sample preparation, is necessary for routine analysis of D-amphetamine in biological samples because of its extensive use in drug research and the interest from toxicologic-forensic investigation. A sensitive, specific and high-throughput bioanalytical procedure is described for the quantitative determination of D-amphetamine in rat blood using MS^3 scan mode on a hybrid triple quadrupole-linear ion trap mass spectrometer (LC-MS/MS/MS). After dilution with water, blood samples were prepared by fully automated protein precipitation with acetonitrile and included an internal standard. Chromatographic separation was achieved on a Waters XTerra C18 column (2.1mm x 30mm, 3.5µm) with gradient elution at a flow rate of 1.0ml/min over a 2min run time. An Applied Biosystems API4000 QTRAP mass spectrometer equipped with turbo ion-spray ionization source was operated simultaneously in MS^3 scan mode for the D-amphetamine and in multiple reaction monitoring (MRM) for the internal standard. The MS/MS/MS ion transition monitored was *m/z* 136.1→119.1→91.1 for the quantitation of D-amphetamine and for the internal standard (rolipram) the MS/MS ion transition monitored was *m/z* 276.1→208.2. The linear dynamic range was measured over the concentration range 0.5-1000ng/ml (r^2 = 0.9991). The method was rugged and sensitive with a lower limit of quantification (LLOQ) of 0.5ng/ml. All the validation data, such as accuracy, precision, and inter-day repeatability, were within the required limits. This technique was successfully applied to evaluate the pharmacokinetics of D-amphetamine in rat. Furthermore, this paper illustrates that the selectivity of the fragmentation pathway (MS^3) may be employed as an alternative approach to significantly improve detection capability in complex situation (e.g., small molecules in complex matrices) rather than increasing time for sample preparation and chromatographic separation

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Forensic Sci Int 2010 **195** (1-3) 108

Cathinone preservation in khat evidence *via* drying

The principle psychoactive component of the khat plant (*Catha edulis*) is cathinone. However, it converts to the less-active substance, cathine, after harvesting and this presents a problem for forensic analysis. The loss of cathinone has serious legal implications because it is a Schedule I controlled substance under federal regulations in the United States. Cathine, on-the-othe-hand, is classified as a Schedule IV substance. A common misconception is that cathinone is highly unstable once the plant is harvested and consequently, might be not detectable following drying and prolonged storage. However, drying the plant material preserves cathinone. Numerous seizures of a dried form of khat (referred to as "graba" in the United States) have been made in recent years. This indicates that drying khat is a viable approach to preserve evidence for both storage and reanalysis. A qualitative and quantitative analysis khat samples seized as dried plant material has demonstrated that khat alkaloids are relatively stable for a period of 3 years. In addition, cathinone remained identifiable when khat was stored at room temperature for over 10 years. Research on green khat (received moist) has revealed that drying the moist leaves at either room temperature or by the application of heat are suitable approaches to preserve cathinone in the dried material. The data illustrate that cathinone persists in dried khat for a time frame of several years. Furthermore, simple drying techniques are an effective means to preserve seized khat evidence for long-term storage

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Rapid Commun Mass Spectrom 2010 **24** (11) 1653

^{13}C and ^{2}H isotope ratios in amphetamine synthesized from benzaldehyde and nitroethane

Research performed previously at National Measurement Institute and by Butzenlechner *et al*. and Culp *et al*. has shown that the δ^2H isotope value of industrial benzaldehyde created by the catalytic oxidation of toluene is profoundly positive, usually in the range +300 per thousand to +500 per thousand. Therefore, synthesis of amphetamine, methylamphetamine and their precursors which begin with such benzaldehyde may be expected to display unusually

positive δ^2H values. Data are provided for δ^{13}C and δ^2H isotope values of 1-phenyl-2-nitropropene synthesized from an industrial source of benzaldehyde, having a positive δ^2H isotope value, by a Knoevenagel condensation with nitroethane. In addition, data are also provided for the δ^{13}C and δ^2H isotope values for amphetamine produced from the resulting 1-phenyl-2-nitropropene. Results were compared with δ^{13}C and δ^2H isotope values derived for an amphetamine sample produced employing a synthetic route not involving benzaldehyde. Furthermore, data are presented for samples of benzaldehyde, 1-phenyl-2-nitropropene and amphetamine that had been produced in an illicit amphetamine laboratory

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Anal Chem 2010 **82** (4) 1358

Fluorescence aptameric sensor for strand displacement amplification detection of cocaine

A new technique for cocaine detection has been produced employing aptamer-target interactions with target-induced strand displacement. New probes have been developed. The hairpin-probe and the single strand-probe (ss-probe), which possess two recognition sequences of cocaine aptamer. Both probes associate with cocaine to form a tripartite complex. A conformational change in the hairpin-probe results in the opening of a hairpin structure and hybridization to primer. With polymerase and the dNTPs, the replication of the single-stranded domain of hairpin-probe activates the process of primer extension. Following conversion of the hairpin-probe into a fully double-stranded form, the ss-probe and cocaine are displaced to bind another hairpin-probe and initiate new amplification cycles. Fluorescence signal generation is observed upon SYBR Green I intercalating into the new DNA double helix. This new approach design facilitates detection of as low as 2 nM cocaine in a closed tube, providing a convenient method for a homogeneous assay. The new method is highly sensitive, selective, and economical in comparison with previously reported cocaine aptameric sensors

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Forensic Sci Int 2010 **194** (1-3) 94

Analysis of hair after contamination with blood containing cocaine and blood containing benzoylecgonine

Blood is a potential source of external contamination of hair in post-mortem work. The present study was performed to examine the amount of drug absorbed into hair which has been contaminated with blood containing either cocaine or BE. Solutions containing 0.05, 0.1, 0.2, 0.5 and 3.0 µg/ml of either cocaine or BE in human blood were prepared. Samples of approximately 3.2g of drug-free hair were contaminated by soaking in the blood solutions for 5 min. Subsequently, they were removed and left at room temperature. Approximately 0.5 g of hair was collected from each of the blood soaked hair samples at 6h, 1, 2, 4 and 7 days after contamination. As each hair sample was collected it was shampoo-washed to preclude additional drug absorption. Using a fully validated procedure described previously, hair samples were analysed in triplicate. In addition, EME and cocaethylene were measured in order to examine whether cocaine or BE was degrading to these compounds. Both cocaine and BE were absorbed into hair in significant concentrations when the concentration in the blood was 0.5 µg/ml or greater and cocaine was more readily than BE. Cocaine broke down to EME (< LOQ) at 0.5 µg/ml and to EME (> LOQ) and BE (< LOQ) at 3.0 µg/ml. There was no evidence of cocaine breaking down to form BE when the blood concentration was 0.5 µg/ml or less. There was no evidence of the BE breaking down in the samples soaked in blood containing BE. When the contamination period increased from 6h to 7 days, the absorption of drug into hair did not increase

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Forensic Sci Int 2010 **194** (1-3) 53

Differentiation of regioisomeric ring-substituted fluorophenethylamines with product ion spectrometry

Electron ionization (EI) of aromatic ring-substituted isomers produces almost identical mass spectra which seriously impairs their analysis. This particularly applies to regioisomeric *meta*- and *para*-ring-substituted compounds which cannot exhibit any ortho-effect reactions so that their differentiation by mass spectrometry is impossible. In addition, *o*-, *m*- and *p*-substituted compounds are insufficiently separated by chromatography due to their very similar retention and this does not allow indisputable identification. Product ion mass spectrometry is a useful approach to differentiate structurally, closely related fluorophenethylamines including *meta*- and *para*-isomers. A series of *N*-alkylated *o*-, *m*- and *p*-fluoroamphetamines and 1-(4-fluorophenyl)butan-2-amines have been synthesized in microscale and analysed by product ion spectrometry. Together, chemical ionization (CI) and product ion spectrometry of hydrogen fluoride loss ions $[M+H-HF]^+$ facilitate precise differentiation of all studied fluoro-substituted phenethylamines without the necessity for prior derivatization. This technique employing submicrogram detection limits provides great benefits for the differentiation between aromatic regioisomeric fluorophenethylamine designer drugs where other procedures for example nuclear magnetic resonance (NMR) spectrometry lack sufficient sensitivity or could fail due to the complexity of mixtures that have to be analyzed

10 Hallucinogens

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Forensic Sci Int 2010 **195** (1-3) 78

Differentiation of methylenedioxybenzylpiperazines (MDBP) by GC-IRD and GC-MS

The substituted benzylpiperazine, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP) and its regioisomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) have virtually identical mass spectra. Mass spectra with differences in relative abundance of some fragment ions result from the perfluoroacylation of the secondary amine nitrogen of these regioisomeric piperazines. However, the spectra did not produce unique fragments which would facilitate specific identification of one regioisomer to the exclusion of the other. Gas chromatographic separation coupled with infrared detection (GC-IRD) on-the-other-hand, produces direct confirmatory data for structural differentiation between the two regioisomers. Mass spectra in combination with the vapor-phase infrared spectra facilitate specific confirmation of each of the regioisomeric piperazines. Underivatized and perfluoroacyl derivative forms of the ring substituted benzylpiperazines were resolved on a 30-m capillary column employing an Rxi-50 stationary phase

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Forensic Sci Int 2010 **194** (1-3) 39

GC-IRD studies on regioisomeric ring substituted methoxy methyl phenylacetones related to 3,4-methylenedioxyphenylacetone

An isobaric relationship (equivalent mass but different elemental composition) is to be found between the methoxy methyl phenylacetones and the controlled precursor substance 3,4-methylenedioxyphenylacetone (3,4-methylenedioxyphenyl-2-propanone; 3,4-MDP-2-P). The ten ring substituted methoxy methyl phenylacetones may be differentiated by capillary gas chromatography on a modified cyclodextrin stationary phase. All ten regioisomeric ketones eluted before 3,4-methylenedioxyphenylacetone. Infrared spectra of the vapor phase produced by the capillary column effluent unambiguously identified 3,4-MDP-2-P from the various methoxy methyl phenylacetones. Furthermore, the methoxy methyl phenylacetones produced unique individual infrared spectra. Infrared absorption frequencies and patterns validated the relative position of the methoxy-group and the acetone side-chain for the regioisomeric ketones

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Rapid Commun Mass Spectrom 2010 **24** (8) 1133

Direct quantification of 11-nor- ^{9}-tetrahydrocannabinol-9-carboxylic acid in urine by liquid chromatography/tandem mass spectrometry in relation to doping control analysis

An accurate and precise method for the quantification of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCA) in urine by liquid chromatography/tandem mass spectrometry (LC/MS/MS) for doping analysis purposes has been developed. The method involves the use of only 200 µl of urine and the use of Δ^9-THCA as internal standard. No extraction procedure is used. The urine samples are hydrolysed using sodium hydroxide and diluted with a mixture of methanol/glacial acetic acid (1:1). Chromatographic separation is achieved using a C8 column with gradient elution. All MS and MS/MS parameters were optimised in both positive and negative electrospray ionisation modes. For the identification and the quantification of THCA three product

ions are monitored in both ionisation modes. The method is linear over the studied range (5-40 ng/ml), with satisfactory intra-and inter-assay precision, and the relative standard deviations (RSDs) are lower than 15%. Good accuracy is achieved with bias less than 10% at all levels tested. No significant matrix effects are observed. The selectivity and specificity are satisfactory, and no interferences are detected. The LC/MS/MS method was applied for the analysis of 48 real urine samples previously analysed with a routine gas chromatography/mass spectrometry (GC/MS) method. A good correlation between the two methods was obtained ($r^2 > 0.98$) with a slope close to 1

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Forensic Sci Int 2010 **195** (1-3) 36

Comparative study of ATR and transflection IR spectroscopic techniques for the analysis of hallucinogenic mushrooms

Psilocin is an active ingredient of some hallucinogenic mushrooms. ATR and transflection spectroscopic techniques were compared for the qualitative analysis of psilocin in hallucinogeic and spiked control mushrooms. Both techniques produced similar data and were in agreement with prior GC/MS analysis of the actual case samples

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Forensic Sci Int 2010 **194** (1-3) 103

Identification and quantitation of 3,4-methylenedioxy-*N*-methylamphetamine (MDMA, Ecstasy) in human urine by ^{1}H NMR spectroscopy. Application to five cases of intoxication

Nuclear magnetic resonance (NMR) spectroscopy of human urine has been used to identify 3,4-methylenedioxy-*N*-methylamphetamine (MDMA, Ecstasy) in five cases of intoxication. A new water suppression technique PURGE (Presaturation Utilizing Relaxation Gradients and Echoes) was employed. A calibration curve was produced with spiked samples. The method gave a linear response (correlation coefficient of 0.992) over the range 0.01-1mg/ml. Subsequently, quantitation of the amount of MDMA present in the samples was carried out. The benefits and reliability of NMR investigations of human urine for cases of intoxication with MDMA are discussed

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Forensic Sci Int 2010 **195** (1-3) 132

Detection of piperonal emitted from polymer controlled odor mimic permeation systems utilizing *Canis familiaris* and solid phase microextraction-ion mobility spectrometry

In terms of odor detection, there is generally a greater variation in the limit of detections (LODs) for canines compared with instruments. Research described in this paper presents an improved protocol for the creation of controlled odor mimic permeation system (COMPS) devices for use as standards in canine training. In addition it discusses the canine detection thresholds of piperonal, a starting material for the illicit drug 3,4-methylenedioxymethamphetamine (MDMA), when exposed to these devices. Furthermore, research is presented of the first-ever reported direct comparison of solid phase microextraction-ion mobility spectrometry (SPME-IMS) to canine detection for the MDMA odorant, piperonal. The research demonstrates the reliability of COMPS devices as low cost field calibrants furnishing a wide range of odorant concentrations for biological and instrumental detectors. The canine LOD of piperonal released from the 100 ng/s COMPS was found to be 1 ng as compared to the SPME-IMS LOD of piperonal in a static, closed system at 2 ng, with a linear dynamic range from 2 ng to 11 ng. The utilization of the COMPS devices should facilitate training that will reduce the detection variability between canines and maintain improved consistency for training purposes. Since both SPME and IMS are field portable technologies. It is expected that this coupled technique will be beneficial as a complement to canine detection for the field detection of MDMA

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Inhal Toxicol 2009 **21** (13) 1108

Cannabis smoke condensate III: The cannabinoid content of vaporised *Cannabis sativa*

Cannabis sativa is a widely employed recreational drug. It is a controlled substance whose possession and use are illegal in most countries of the world. Considering the legal restrictions on the possession and use of *C. sativa*, relatively little research on the medicinal qualities of this plant has been conducted. However, interest in the medicinal uses of this plant has increased in the last decades. For medicinal purposes, methods of administration are primarily *via* oral ingestion, smoking and recently, inhalation through vaporization. During this research, the commercially available Volcano vaporizing device was compared with cannabis cigarette smoke. Cannabis smoke and vapor (obtained at different temperatures) were quantitatively analyzed by high-performance liquid chromatography (HPLC). Also, different quantities of cannabis material were also tested with the vaporizer. The cannabinoids:by-products ratio in the vapor obtained at 200 °C and 230 °C was significantly higher than in the cigarette smoke. The worst ratio of cannabinoids:by-products was produced from the vaporized cannabis sample at 170 °C

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J Forensic Sci 2010 **55** (3) 605

Isolation and identification of three by-products found in methylamphetamine synthesized by the Emde route

This article describes the isolation and structural elucidation of three compounds produced during the synthesis of methylamphetamine by the so-called "Emde" procedure. The "Emde" procedure involves the preparation of the intermediate chloropseudoephedrine or chloroephedrine from ephedrine or pseudoephedrine, respectively. The intermediates are then reduced to methylamphetamine with hydrogen under pressure in the presence of a catalyst. The by-product compounds were isolated from methylamphetamine by column chromatography and liquid chromatography (LC). Proton nuclear magnetic resonance spectroscopy (^{1}H NMR), carbon nuclear magnetic resonance spectroscopy (^{13}C NMR), and nanospray quadrupole-time of flight-mass spectrometry (Q-TOF-MS) were used to identify them as two stereoisomers of the compound *N*,*N*'-dimethyl-3,4-diphenylhexane-2,5-diamine and *N*-methyl-1-{4-[2-(methylamino)propyl]phenyl}-1-phenylpropan-2-amine

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J Forensic Sci 2010 **55** (2) 531

Quantitative analysis of -hydroxybutyrate at endogenous concentrations in hair using liquid chromatography tandem mass spectrometry

A method capable of quantifying endogenous concentrations of γ-hydroxybutyrate (GHB) in human head hair was developed and validated using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). Hair was digested under alkaline conditions, and γ-hydroxybutyrate was isolated using liquid-liquid extraction. Liquid chromatography tandem mass spectrometry was performed using atmospheric pressure chemical ionization in the negative mode, multiple reaction monitoring, and deuterated internal standard (γ-hydroxybutyrate-D_6). Linearity was observed between 0.1 and 100 ng/mg γ-hydroxybutyrate (R^2 = 1.000). The limits of detection and quantitation in human hair were 0.2 and 0.4 ng/mg, respectively. Accuracy at 2 ng/mg and 10 ng/mg was determined to be 97% and 94%, and intra-assay CVs at these concentrations were 5.2% and 7.4% (n = 4). β-hydroxybutyrate (BHB), α-hydroxybutyrate, γ-butyrolactone, and 1,4-butanediol did not produce an interference, and there was negligible ion suppression or enhancement from the matrix

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Anal Chim Acta 2010 **658** (2) 187

Direct detection of ^{9}-tetrahydrocannabinol in saliva using a novel homogeneous competitive immunoassay with fluorescence quenching

A homogeneous competitive immunoassay based on fluorescence quenching induced by fluorescence resonance energy transfer (FRET) has been developed for the detection of the major active component of cannabis, Δ^9-tetrahydrocannabinol (THC) in aqueous samples. The fluorescence of anti-THC-antibody, labeled with fluorescence dye DY-481XL, may be quenched after binding to the THC-BSA-quencher conjugate (bovine serum albumin coupled with THC and another fluorescence dye, DYQ-661, as quencher). The quenching effect is inhibited when the antibodies bind to free THC in aqueous sample, thus competing for binding sites with the THC-BSA-quencher conjugate. The level of the inhibition corresponds with the concentration of THC in the samples. The assay principle is simple and the test duration is within 10 min. The detection limit for THC in buffer was 2 ng/ml. In pooled saliva samples a detection limit of 50 ng/ml was accomplished

11 Narcotics

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Forensic Sci Int 2010 **195** (1-3) 68
Studies on 1-(2-phenethyl)-4-(*N*-propionylanilino)piperidine (fentanyl) and related compounds VII. Quantification of -methylfentanyl metabolites excreted in rat urine
Designer drugs, chemically modified controlled drugs, are globally available. During the 1980s, the dominant drugs of abuse were modifications of fentanyl formed by methylation of both the α-position of its phenethyl group (α-methylfentanyl) and the 3-position of its piperidine ring (3-methylfentanyl). There have been numerous analytical methods for fentanyl and its analogues. In addition, many studies of its metabolism and major metabolites have been reported. However, minor metabolites that disclosed the original injection compound(s) were not included in these studies. Recently, the structures of four novel and minor metabolites that reflect α-methylfentanyl have been described. This research quantifies excretion of these compounds in the 96 h following peroral injection to rats of 3mg/day and urine collection every 24h. Major metabolites were as for fentanyl, with approximately 24% of α-methylfentanyl excreted as nor-fentanyl and 15% as ω, ω-1 hydroxypropiony nor-fentanyl up to 72 h post-injection. The novel metabolites were totally excreted within 48 h of injection and composed 2-3% of the total metabolites. The primary metabolite nor-fentanyl was detected up to 72 h after injection

12 Forensics

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J Forensic Sci 2010 **55** (2) 508
Comparison of frequentist methods for estimating the total weight of consignments of drugs
A topic in forensic statistics is the estimation of the total weight of consignments of drugs based on subsamples of which a certain fraction may not contain drugs at all. The frequentist approach to this concentrates on obtaining confidence intervals for the total weight, based on estimation of the fraction of drugs and the mean and variance of the weights of drug units. The current study shows that the resulting confidence intervals are basically unreliable, since they are based on an underestimation of the variation of the underlying statistical process. Two alternatives are given that yield asymptotically correct results. These are not reliable for small subsamples either, though, because of the inherent multimodal behaviour of the sample mean. In cases where a relatively large fraction of the consignment contains no drugs, the confidence intervals reported in the literature should not be used in practice

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J Forensic Sci 2010 **55** (2) 457
Postmortem blood concentrations of *R*- and *S*-enantiomers of methadone and EDDP in drug users: Influence of co-medication and P-glycoprotein genotype
We investigated toxicological and pharmacogenetic factors that could influence methadone toxicity using postmortem samples. *R*- and *S*-methadone were measured in femoral blood from 90 postmortem cases, mainly drug users. The *R*-enantiomer concentrations significantly exceeded that of the *S*-enantiomers (Wilcoxon's test, $p < 0.001$). The samples were divided into four groups according to other drugs detected (methadone only, methadone and strong analgesics, methadone and benzodiazepines, or methadone and other drugs). There was no significant difference in any of the *R*-methadone/total methadone ratios among the four groups. The median *R/S* ratio was 1.38, which tends to be higher than that reported for the plasma of living subjects. In addition, we investigated whether small nucleotide polymorphisms in the *MDR1* gene that encode the drug transporter P-glycoprotein were associated with the concentrations of *R*- and *S*-methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine. No significant association was detected

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J Forensic Sci 2010 **55** (3) 723
DETECHIP : A sensor for drugs of abuse
The design and preliminary characterization of a novel sensor for drugs of abuse, DETECHIP®, is described in this proof-of-concept note. Combining both colorimetric and fluorimetric assays, DETECHIP® is suitable for lab and field use. More than a conventional spot test which provides a single "yes or no" answer, DETECHIP® provides twenty responses for a more complete characterization of suspect material. This is accomplished by visually noting colorimetric and fluorescent changes of carefully selected dyes upon the addition of test analytes, including drugs of abuse, with respect to controls. Color and fluorescence changes are recorded numerically so that a 20 digit identification code can be constructed for comparison of test analytes and known compounds. DETECHIP® is applicable to a variety of drugs, both plant-derived and synthetic, addressing the need to use several different spot tests simultaneously for a single sample

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Forensic Sci Int 2010 **194** (1-3) 28
Gender-related differences in the pharmacokinetics of opiates
In previous research, gender-related differences in a number of aspects of the pharmacology of opiates, including their analgesic activity, stimulative properties and generation of physical dependence have been reported. The current paper examines whether male-female differences exist in the blood and brain levels of opiates as a result of intraperitoneal injection to male and female Wistar rats. Analysis was performed 5, 15, 45 and 120 min after the animal treatment with seized heroin. A gas chromatography-mass spectrometry (GC-MS) method was developed to measure opiate alkaloids in blood and brain regions known for their high concentration of mu-opiate receptors (cortex, brainstem, amygdala and basal ganglia). Greatest levels of opiates in blood of animals of both genders were discovered in the second measurement time (15 min). Values in males were significantly higher and suggests a faster passage of the opiates from blood to brain tissue in female animals. The largest amount of opiates in the brain tissue of female animals was measured at 15 min and with male animals at 45 min after the treatment. This also suggets a faster distribution of opiates from blood to brain in female compared with male rats. In animals of both genders, the highest proportion of opiates was found in the basal ganglia. The data indicate the possibility of selecting this part of the brain tissue of both males and females as a representative sample for identifying and assessing contents of opiates

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J Anal Toxicol 2010 **34** (1) 53
Preliminary gas chromatography with mass spectrometry determination of 3,5-dimethoxyphenol in biological specimens as evidence of *Taxus* poisoning
Taxus baccata is a widely distributed tree commonly known as yew. Its leaves contain high levels of the cardiotoxic alkaloids, taxine A and taxine B and have been associated with cases of fatal intoxication. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS-MS) methods were employed to confirm a case of *Taxus* fatal poisoning, hypothesized by the forensic autopsy. GC-MS was employed to determine derivatized 3,5-dimethoxyphenol, a cyanogenic aglylactone regarded as a marker of Taxus poisoning and present in all Taxus species. LC-MS-MS detection of taxine B and isotaxine B validated the ingestion of cardiotoxic alkaloids in *Taxus baccata* leaves

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J Mass Spectrom 2010 **45** (4) 391
Qualitative screening for volatile organic compounds in human blood using solid-phase microextraction and gas chromatography-mass spectrometry
A fast and simple screening procedure using solid-phase microextraction and gas chromatography-mass spectrometry (SPME-GC-MS) in full-scan mode for the determination of volatile organic compounds (VOC) is presented. The development of a fast and simple screening technique for the simultaneous determination of various volatiles is of great importance, because of their widespread use, frequent occurrence in forensic toxicological questions and the fact that there is often no hint on involved substances at the crime scene. To simulate a screening procedure, eight VOC with different chemical characteristics were chosen (isoflurane, halothane, hexane, chloroform, benzene, isooctane,

toluene and xylene). To achieve maximum sensitivity, variables that influence the SPME process, such as type of fiber, extraction and desorption temperature and time, agitation and additives were optimized by preliminary studies and by means of a central composite design. The limits of detection and recoveries ranged from 2.9 µg/l (xylene) to 37.1 µg/l (isoflurane) and 7.9% (chloroform) to 61.5% (benzene), respectively. This procedure may be used to answer various forensic and toxicological questions. The short time taken for the whole procedure may make its eventual adoption for routine analysis attractive

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Forensic Sci Int 2010 **194** (1-3) 60

Detection of acute fentanyl exposure in fresh and decomposed skeletal tissues part II: The effect of dose-death interval

An automated enzyme-linked immunosorbent assay (ELISA) has been employed to determine the effects of dose-death interval on the detection of acute fentanyl exposure in fresh and decomposed skeletal tissues (marrow and bone). Fentanyl was administered acutely to rats (n = 14) at a dose of 0 (n = 2) or 60 µg/kg (n = 12) by intraperitoneal injection. Rats were euthanized within 20, 45, 135, or 225 min. Femora and tibiae were extracted from the fresh corpses and marrow was isolated from the femoral and tibial medullary cavities. Remains were allowed to decompose outdoors to the point of complete skeletonization. Vertebrae, pelvi and miscellaneous (humeri and scapulae) were recovered for analysis. Bones were cleaned in alkaline solution and then ground into a fine powder. Marrow was homogenized in alkaline solution. Fentanyl was extracted from ground bone with methanolic solution. Extracts were adjusted to pH 6 and analyzed by ELISA. Perimortem heart blood was also collected and diluted with phosphate buffer prior to screening by ELISA. Effects of tissue type on ELISA response were investigated through determination of binary classification test sensitivity and the relative decrease in absorbance (%DA, drug-positive tissues *vs*. drug-free controls). Generally, the %DA varied significantly between extracts from different skeletal tissues at a given dose-death interval, according to the general order of marrow>decomposed bone>fresh bone. Binary classification test sensitivity values for fentanyl in marrow, fresh epiphyseal (femoral and tibial) bone, fresh diaphyseal (femoral and tibial) bone, decomposed vertebrae, decomposed pelvic bone, and decomposed miscellaneous bone were 67-100%, 0-33%, 0-33%, 0-67%, 0-67% and 0-33%, respectively, over all dose-death intervals. Whereas group mean %DA values showed a strong negative correlation with dose-death interval in marrow, fresh epiphyseal bone, decomposed vertebrae, pelvic and miscellaneous bone (r = -0.989, -0.930, -0.955, -0.903, and -0.974, respectively), the high variability in both fresh and decomposed bone impaired differentiation of the dose-death intervals based on %DA value alone. In general, the data suggest that the type of skeletal tissue sampled may not be as important as the amount of residual marrow remaining in skeletonized remains.

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Forensic Sci Int 2010 **195** (1-3) 73

Buprenorphine and major metabolites in blood specimens collected for drug analysis in law enforcement purposes

The quantification of buprenorphine (BUP), norbuprenorphine (NBUP), buprenorphine-3-β-D-glucuronide (BUPG) and norbuprenorphine-3-β-D-glucuronide (NBUPG) in serum samples was achieved with a liquid chromatographic/electrospray ionization tandem mass spectrometric technique which was both developed and validated. Liquid-liquid extraction was employed as pre-treatment of BUP and NBUP. Glucuronides were favourably isolated by solid phase extraction. Separation in 2 separate runs (2 x 5 min) was accomplished with isocratic elution. The procedure was employed with 20 authentic serum specimens collected for law enforcement purposes where BUP intake had been implied. The parent drug was not detectable in half of the specimens at a lower limit of detection of 0.2 ng/ml, whereas NBUP could be determined from all but one sample. NBUPG is the major metabolite present. It could be identified in addition to BUPG in all samples under analysis. In authentic specimens it might be advisable to audit BUP metabolites in addition to the parent drug

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J Planar Chromatogr Mod TLC 2009 **22** (6) 449

A new thin-layer chromatographic method for analysis of zolpidem and zopiclone

No abstract available

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Forensic Sci Int 2010 **194** (1-3) 77

Postmortem molecular screening for mutations in ryanodine receptor type 1 (RYR1) gene in psychiatric patients suspected of having died of neuroleptic malignant syndrome

It is difficult to perform a postmortem diagnosis of neuroleptic malignant syndrome (NMS) because the clinical symptoms preceding death are frequently not available. Malignant hyperthermia (MH) is a catastrophic, life-threatening hypermetabolic syndrome induced by certain anesthetics. Ryanodine receptor type 1 (RYR1) gene mutations are accepted to be involved in susceptibility to MH. Elevated body temperature is a feature of both NMS and MH and this has resulted in the suggestion that NMS may be a neurogenic form of MH. Eleven cases suspected of having elevated body temperature and NMS at death were investigated. Causes of death could not be determined by autopsy examinations. Possible mutations of the RYR1 gene were analysed and two mutations (R4645Q and A612T) were identified. The R4645Q mutation has previously been reported in MH patients. However, five heterozygous mutations were also found in 400 Japanese control alleles. The other mutation was novel, and was not found in the same control alleles. The data provide the first successful identification of RYR1 mutations in psychiatric patients suspected at autopsy of having died of NMS. However, the association between RYR1 gene mutations and cause of death in psychiatric patients suspected of dying of NMS remains unclear

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Forensic Sci Int 2010 **194** (1-3) e17

Multidrug poisoning involving nicotine and tramadol

Tramadol is a centrally acting analgesic employed to treat moderate to severe acute or chronic pain. Nicotine, a lipid-soluble alkaloid, is one of the most widely distributed drugs in modern society. A fatal case of multidrug poisoning involving tramadol and nicotine is described. A 46-year-old man was discovered dead in his bed. A suicide note was found near his body. He had 25 transdermal nicotine patches attached to his thorax and abdomen. Two half emptied bottles were found on the bedside table. Toxicological analysis proved that they contained tobacco and nicotine plus other drugs such as diphenhydramine. Upon autopsy, areas of fresh and old myocardial infarction in addition to diffuse pulmonary congestion and edema were noted. In femoral venous blood, the tramadol concentration was 6.6 µg/ml whereas levels of nicotine and its primary metabolite cotinine were determined to be 0.6 and 2.0 µg/ml respectively. The cause of death was determined to be cardiorespiratory failure induced by the additive effects of tramadol and nicotine shortly following consumption

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Forensic Sci Int 2010 **195** (1-3) e19

Death due to ingestion of nicotine-containing solution: Case report and review of the literature

Nicotine is a lipid-soluble alkaloid obtained from the dried leaves of *Nicotiana*. It is most usually encountered in tobacco derivatives produced for smoking, chewing or sniffing. It is also present in a limited number of pesticides. Whereas nicotine is one of the most toxic drugs of abuse, it has rarely results in fatalities. Sudden death may result from cardiovascular arrest, respiratory muscle paralysis and/or central respiratory failure. A 42-year-old man was discovered dead by his wife. He was found on the floor next to a box containing many empty bottles of beer and vodka. Some labeled chemical bottles discovered at the scene contained various substances, including nicotine and brucine. Gross examination of the organs at autopsy produced no specific findings. Toxicological analysis failed to disclose any lethal toxic agents other than a high concentration of nicotine and its primary metabolite cotinine in femoral venous blood (2.2 µg/ml). Blood alcohol was estimated to be 2.1 g/l in femoral venous blood.To date, there is a dearth of fatal cases of nicotine poisoning has been reported in the literature

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J Anal Toxicol 2010 **34** (1) 39

Beating the system: A study of a creatinine assay and its efficacy in authenticating human urine specimens

The authenticity of urine specimens submitted for illicit drug screening is

frequently validated by the creatinine concentration. This paper examines creatinine screening of donor urine specimens as a technique to discover substituted and/or tampered specimens. Creatinine assays were performed on animal urine, fruit juices, and urine from creatine-supplemented subjects with a modified version of the Jaffe reaction. Creatinine concentrations were measured with a chemistry-immunoanalyzer. Data demonstrate that urine of common domestic pets, including cats, dogs, and horses, has similar creatinine values to that of humans. Most fruit juices showed no detectable creatinine and the few that did had little resemblance to urine. Creatine supplementation of donorsfailed to facilitate an effective method of elevating the creatinine concentration in urine when attempting to flush out water-soluble drugs in the body. Consequently, thecreatinine assay was of benefit in the detection of some but not all adulterated urine specimens

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J Anal Toxicol 2010 **34** (1) 26

Postmortem acidification of blood/organs induces an increase in flecainide concentration in cardiac blood and the contribution of the lungs to this increase

Postmortem acidification of blood and its effects towards raised flecainide concentrations in cardiac blood were investigated following intravenous administration to rabbits. Antemortem peripheral blood was collected at 15 min after which the rabbits were sacrificed. Blood and organs were collected either immediately or 24 h after death or alternatively immediately or 24 h following cardiac massage. Postmortem left/right cardiac blood and organs revealed lower pH than antemortem blood. Furthermore, flecainide concentrations in all postmortem blood specimens were higher than those taken antemortem. Raised flecainide concentrations in cardiac blood were further increased by postmortem cardiac massage and postmortem interval. In perfusion experiments with lung and heart, even when the flecainide concentration in inflow was kept constant, outflow concentrations were 2- to 3-fold higher than in inflow when inflow pH changed from 7.4 to 5.5. However, flecainide concentration in outflow declined immediately and then remained low when the pH of the perfusate changed from 5.5 to 7.4. Data show that flecainide accumulates in the lungs before death. In addition, the accumulated flecainide is liberated into the blood following postmortem acidification of blood/organs

13 Alcohol

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Microchim Acta 2010 **168** (1-2) 141

A fluorescent chemical sensor for ethanol determination in alcoholic beverages

The fluorescent probe 5,10,15,20-tetraphenyl porphyrin (TPP). has be utilised for the production of a sensor for ethanol. The quenching of the fluorescence of TPP by ethanol as a result of electrostatic attraction produces the measurable signal. Within the concentration range from 1 to 75 vol.% ethanol, the sensor responds linearly. It was applied to the determination of ethanol in various kinds of wines and whisky

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Rapid Commun Mass Spectrom 2010 **24** (12) 1737

Comparison of analytical approaches for liquid chromatography/mass spectrometry determination of the alcohol biomarker ethyl glucuronide in urine

Official guidelines originating from a European Union directive regulate requirements for analytical methods used to identify chemical compounds in biological matrices. This study compared different liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) and tandem mass spectrometry (LC/ESI-MS/MS) procedures for accurate determination of the conjugated ethanol metabolite and alcohol biomarker ethyl glucuronide (EtG) in urine, and the value of combined EtG and ethyl sulfate (EtS) measurement. Analysis was carried out on 482 urines following solid-phase extraction (SPE) sample cleanup or using direct injection of a diluted sample. SPE combined with LC/MS/MS was demonstrated to be the most selective and sensitive method and was chosen as reference method. The EtG results by different methods showed good correlation (r = 0.96-0.98). When comparing five reporting limits for EtG in the range 0.10-1.00 mg/L, the overall agreement with the reference method (frequency of true positives plus true negatives) was 82-97% for direct-injection LC/MS/MS, 90-97% for SPE-LC/MS, 86-98% for direct-injection LC/MS, and 86-98% for direct-injection LC/MS analysis of EtG and EtS. Most deviations were attributable to uncertainty in quantitation, when the value was close to a cutoff but the respective results were slightly above and below, or vice versa, the critical limit. However, for direct-injection LC/MS/MS, despite earning 4 identification points, equally many negative results were due to a product ion ratio outside the +/-20% deviation accepted by the guidelines. These results indicate that the likelihood of different analytical methods to provide reliable analytical results depends on the reporting limit applied

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Forensic Sci Int 2010 **194** (1-3) 97

Relationship between blood and urine alcohol concentrations in apprehended drivers who claimed consumption of alcohol after driving and without supporting evidence

Many people suspected of driving under the influence of alcohol (DUIA) are not apprehended when stopped or involved in an incident but some time after the driving. This provides them with the opportunity to claim that they consumed alcohol after the time of driving or after they were involved in an accident. Alleged post-offence drinking is difficult for the prosecution to disprove. This frequently results in the DUIA charge being dropped or the person is acquitted should the case goes to trial. The routine procedure of sampling and measuring the concentration of alcohol in blood (BAC) and urine (UAC) and calculating urine/blood ratios (UAC/BAC) and the changes in UAC between two successive voids furnishes useful information to support or challenge alleged drinking after driving. In this study, a retrospective case series of DUIA offenders (n = 40) is examined in which half had supporting evidence of a subsequent drink (eye witness or police reports) and in the other half where no such evidence existed except for the suspect's admission. Where there was supporting evidence of a subsequent drink, the UAC/BAC ratio for the first void was close to or less than unity (mean 1.04, median 1.08, range 0.54-1.21) and the UAC increased by 0.21 g/l (range 0.02-0.57) between the two voids. In the absence of supporting evidence of post-offence drinking, the mean UAC/BAC ratio was 1.46 (range 1.35-1.93) for the first void, verifying that absorption and distribution of alcohol in all body fluids and tissues was complete. In such instances, the UAC between successive voids decreased by 0.25 g/l on average (range 0.10-0.49), indicating the post-absorptive phase of the BAC curve. Long experience by the authors of investigating claims of post-offence drinking has resulted in the conclusion that in the vast majority of cases this lacks any substance and is simply a last resort by DUIA offenders to evade justice. In the absence of supporting evidence (eye witness, police reports, etc.) of post-offence drinking, the courts are encouraged to ignore this excuse by the defence

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Forensic Sci Int 2010 **194** (1-3) 34

Computer assisted modeling of ethyl sulfate pharmacokinetics

In a drinking experiment with 12 volunteers, the concentration-time-courses of ethyl sulfate (EtS) and ethanol were simulated and matched with the experimental data. The same mathematical model previously used for ethyl glucuronide (EtG) was employed to describe the concentration-time-courses. The following assumptions and simplifications were employed as the basis of the kinetic model: a velocity constant k_{form} for the first order formation of ethyl sulfate from ethanol and an exponential elimination constant k_{el}. The mean values (and standard deviations) obtained for k_{form} and k_{el} were 0.00052/h (0.00014) and 0.561/h (0.131), respectively. By employing the ranges of these parameters it is possible to estimate minimum and maximum serum concentrations of EtS based on stated ethanol doses and drinking times. A comparison of estimated and actual concentrations may prove the plausibility of alleged ethanol consumption and add evidence to the retrospective calculation of ethanol concentrations based on EtG concentrations

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Rapid Commun Mass Spectrom 2010 **24** (7) 1066

Kinetics of ethanol decay in mouth- and nose-exhaled breath measured on-line by selected ion flow tube mass spectrometry following varying doses of alcohol

A study has been carried out of the decay of ethanol in mouth-exhaled and nose-exhaled breath of two healthy volunteers following the ingestion of various doses of alcohol at different dilutions in water. Concurrent analyses of

sequential single breath exhalations from the two volunteers were carried out using selected ion flow tube mass spectrometry, SIFT-MS, on-line and in real time continuously over some 200 min following each alcohol dose by simply switching sampling between the two volunteers. Thus, the time interval between breath exhalations was only a few minutes, and this results in well-defined decay curves. Inspection of the mouth-exhaled and nose-exhaled breath data shows that mouth contamination of ethanol diminished to insignificant levels after a few minutes. The detailed results of the analyses of nose-exhaled breath show that the peak levels and the decay rates of breath ethanol are dependent on the ethanol dose and the volume of ethanol/water mixture ingested. From these data, both the efficiency of the first-pass metabolism of ethanol and the indications of gastric emptying rates at the various doses and ingested volumes have been obtained for the two volunteers. Additionally and simultaneously, acetaldehyde, acetic acid and acetone were measured in each single breath exhalation. Acetaldehyde, the primary product of ethanol metabolism, is seen to track the breath ethanol. Acetic acid, a possible secondary product of this metabolism, was detected in the exhaled breath, but was shown to largely originate in the oral cavity. Breath acetone was seen to increase over the long period of measurement due to the depletion of nutrients

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J Forensic Sci 2010 **55** (3) 767

Proficiency testing as a basis for estimating uncertainty of measurement: Application to forensic alcohol and toxicology quantitations

While forensic laboratories will soon be required to estimate uncertainties of measurement for those quantitations reported to the end users of the information, the procedures for estimating this have been little discussed in the forensic literature. This article illustrates how proficiency test results provide the basis for estimating uncertainties in three instances: (i) For breath alcohol analyzers the interlaboratory precision is taken as a direct measure of uncertainty. This approach applies when the number of proficiency tests is small. (ii) For blood alcohol, the uncertainty is calculated from the differences between the laboratory's proficiency testing results and the mean quantitations determined by the participants; this approach applies when the laboratory has participated in a large number of tests. (iii) For toxicology, either of these approaches is useful for estimating comparability between laboratories, but not for estimating absolute accuracy. It is seen that data from proficiency tests enable estimates of uncertainty that are empirical, simple, thorough, and applicable to a wide range of concentrations

14 Tobacco

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J Chromatogr B 2010 **878** (1) 107

Determination of nicotine and cotinine in human serum by means of LC/MS

An HPLC electrospray ionisation mass spectrometry procedure with solid-phase extraction sample preparation has been developed for the quantitative determination of nicotine and cotinine in human serum in volunteers. Measured concentrations of nicotine and cotinine were employed in respect of control of smoking behaviour. A Waters X-Bridge-column and a SSQ 7000 single quadropole mass spectrometer with a TSP liquid chromatographic system were utilised. The technique incorporates simple and robust sample preparation. The assay has been demonstrated to exhibit adequate sensitivity for this application. The limits of quantification were 5 and 2ng/ml for cotinine and nicotine, respectively

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J Chromatogr A 2010 **1217** (3) 307

Determination of pyridine, 2-picoline, 4-picoline and quinoline from mainstream cigarette smoke by solid-phase extraction liquid chromatography/electrospray ionization tandem mass spectrometry

A new reversed-phase liquid chromatography-electrospray ionization tandem mass spectrometric method (RP-HPLC-ESI-MS/MS) for simultaneous determination of pyridine, 2-picoline, 4-picoline and quinoline from mainstream cigarette smoke has been developed and validated. Liquid-liquid extraction and subsequent solid-phase extraction was employed to extract the target analytes from cigarette smoke. Baseline chromatographic separation was accomplished by use of a Zorbax SB-Aq (4.6x150 mm, 5 µm) column in gradient chromatographic conditions with acetonitrile and ammonium acetate buffer as mobile phases. Popular commercially available Indian brand filtered and non-filtered cigarettes were analyzed. Identification of each compound was achieved by chromatographic retention times, analyte specific fragmentation patterns and relative peak area ratios of two product/precursor ion pairs. The limit of detection of this method ranged from 1.74 to 14.32 ng/cig using an injection volume of 20 µl. The reproducibility of this technique was excellent. In addition, better standard deviations were produced in comparison with literature reported values for these ccompounds. RSD value was less than 9% for all analytes

15 Homeland Security

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Anal Chem 2010 **82** (3) 798

Ricin activity assay by direct analysis in real time mass spectrometry release detection of adenine release

Biotoxin activity assays usually employ multistep sample preparation, multicomponent reactions, multistep analysis, or a combination thereof. In this paper a single-step, real-time ricin activity assay that requires little or no sample preparation and employs direct analysis in real time mass spectrometry is described. The release of adenine from the inhomogeneous substrate herring sperm DNA by ricin was estimated to be 53 +/- 2 pmol adenine per picomole of ricin per hour. This method may be readily adapted to any enzyme for which a reactant or product of low molecular weight (up to approximately 600) may be identified

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Rapid Commun Mass Spectrom 2010 **24** (11) 1617

Desorption electrospray ionization mass spectrometric analysis of organophosphorus chemical warfare agents using ion mobility and tandem mass spectrometry

Desorption electrospray ionization mass spectrometry (DESI-MS) has been applied to the direct analysis of sample media for target chemicals, including chemical warfare agents (CWA), without the need for additional sample handling. During the present study, solid-phase microextraction (SPME) fibers were used to sample the headspace above five organophosphorus CWA, *O*-isopropyl methylphosphonofluoridate (sarin, GB), *O*-pinacolyl methylphosphonofluoridate (soman, GD), *O*-ethyl *N*,*N*-dimethyl phosphoramidocyanidate (tabun, GA), *O*-cyclohexyl methylphosphonofluoridate (cyclohexyl sarin, GF) and *O*-ethyl *S*-2-diisopropylaminoethyl methyl phosphonothiolate (VX) spiked into glass headspace sampling vials. Following sampling, the SPME fibers were introduced directly into a modified ESI source, enabling rapid and safe DESI of the toxic compounds. A SYNAPT HDMS instrument was used to acquire time-aligned parallel (TAP) fragmentation data, which provided both ion mobility and MS^n (n = 2 or 3) data useful for the confirmation of CWA. Unique ion mobility profiles were acquired for each compound and characteristic product ions of the ion mobility separated ions were produced in the Triwave transfer collision region. Up to six full scanning MS^n spectra, containing the $[M + H]^+$ ion and up to seven diagnostic product ions, were acquired for each CWA during SPME fiber analysis. A rapid screening approach, based on the developed methodology, was applied to several typical forensic media, including Dacron sampling swabs spiked with 5 µg of CWA. Background interference was minimal and the spiked CWA were readily identified within one minute on the basis of the acquired ion mobility and mass spectrometric data

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Anal Bioanal Chem 2010 **396** (3) 1213

Monitoring the hydrolysis of toxic organophosphonate nerve agents in aqueous buffer and in bicontinuous microemulsions by use of diisopropyl fluorophosphatase (DFPase) with ^{1}H-^{31}P HSQC NMR spectroscopy

Diisopropyl fluorophosphatase (DFPase, EC 3.1.8.2) from the squid *Loligo vulgaris* is an enzyme which effectively catalyzes the hydrolysis of diisopropyl fluorophosphate (DFP) and a number of organophosphorus nerve agents, including sarin, soman, cyclosarin, and tabun. To date, determination of kinetic data has been accomplished by use of methods including pH-stat titration, ion-selective electrodes, and a recently introduced procedure based on *in situ* Fourier-transform infrared (FTIR) spectroscopy. In this paper, 1D ^{1}H-^{31}P HSQC NMR spectroscopy is employed as a new technique for real-time quantification of the hydrolysis of toxic organophosphonates by DFPase. The

protocol was employed for the agents sarin (GB), soman (GD), and cyclosarin (GD). However, it may also be used for V-type nerve agents such as VX. In addition to buffered aqueous solutions the approach was employed to determine enzymatic activities in a biodiesel-based bicontinuous microemulsion that represents an example of complex decontamination media where other established procedures frequently fail. The protocol is non-invasive and requires only limited manual handling of small volumes of liquid (700 µl). This facilitates work safety when handling highly toxic organophosphorus compounds. Limits of detection were slightly below 100 µmol/l on a 400 MHz spectrometer with 16 FIDs added for a single time frame. The technique is not restricted to DFPase but may be employed with other phosphotriesterases, for example paraxonase (PON), and even reactive chemicals, for example oximes and other nucleophiles, provided that the reaction components are compatible with NMR

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Anal Lett 2010 **43** (3) 417

Comparison of organic solvents used for the determination of mustard gas hydrolysis products in urine samples using gas chromatography-mass spectrometry

A gas chromatography-mass spectrometry procedure was optimized to determine *N*-ethyldiethanolamine (EDEA) and *N*-methyldiethanolamine (MDEA) in urine samples. Analytes were derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and the most suitable organic solvent was investigated. Recoveries were greatest with acetonitrile and dimethylsulphoxide. In spiked samples (100 ng/ml), recoveries were 108 +/- 5.7% for EDEA and 97 +/- 6.4 for MDEA. The limit of detection (LOD, *S/N* = 3) values were 0.5 ng/ml and 0.4 ng/ml for EDEA and MDEA, respectively. The precision of the procedure in terms of RSD was between 5-8%.

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Bunseki Kagaku 2010 **59** (1) 65

Evaluation of detection performance of portable aspiration-type ion mobility spectrometer with seven detection cells for chemical warfare agents (Japanese, English Abstract)

A portable ^{241}Am ionization aspiration-type ion mobility spectrometer (M90-D1-C, Environics Oy) was tested with nerve gases, blister agents, blood agents, choking agents and related compounds. Vapors of nerve gases, sarin, soman, tabun, cyclohexylsarin were recognized as "NERVE" after several seconds of sampling. Limits of detection (LOD) were < 0.3 mg/m. Vapors of the blister agents (mustard gas and lewisite 1) and the blood agents (hydrogen cyanide and cyanogen chloride) were recognized as "BLISTER" . The LODs were < 2.4 mg/m^3 and > 415 mg/m^3, respectively. The vapor of chlorine was recognized as "BLOOD". It had an LOD of 820 mg/m^3. Vapors of nitrogen mustard 3 and chlorpicrin were recognized as different alarm classes, depending on their concentrations. However, vapors of nitrogen mustard 1, 2 and phosgene did not result in any alarm. In respect of interference, vapors of nerve gas simulants, dimethylmethylphosphonate, trimethylphosphate, triethylphosphate, diisopropylfluorophosphate, blister agent simulants, 2-chloroethylethylsulfide, 1,4-thioxane, 2-mercaptoethanol, and 20 organic solvents within 38 solvents examined registered false-positively. Patterns of detection sensor channel response values of 6 ion mobility cells and semiconductor cell were compared with the situation of the alarm against chemical-warfare agents

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Analyst 2010 **135** (2) 368

Novel pyrenehexafluoroisopropanol derivative-decorated single-walled carbon nanotubes for detection of nerve agents by strong hydrogen-bonding interaction

The fabrication of hybridized single-walled carbon nanotube (SWNT) device based on novel sensing material *N*-4-hexafluoroisopropanolphenyl-1-pyrenebutyramide (HFIPP) *via* a simple procedure is reported for the first time. Strong hydrogen-bonding occurs between HFIPP and dimethyl methylphosphonate (DMMP) (simulant of nerve agent sarin). Therefore, the HFIPP-decorated SWNT device has been used to detect DMMP. The detection limit achieved with the sensor was as low as 50 ppb. In addition, the response and reproducibility may be presented clearly even at a very low concentration of DMMP. The excellent sensitivity and selectivity of the hybridized SWNT-HFIPP device indicates that it possesses great potential for the detection of explosives and nerve agents. To facilitate an investigation of the sensing mechanism of SWNT-HFIPP for DMMP, control electrical and fluorescent experiments were performed and are discussed in the present paper

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J Forensic Sci 2010 **55** (3) 801

Denatured ricin can be detected as native ricin by immunological methods, but nontoxic *in vivo*

Ricin is a glycoprotein from *Ricinus communis* seeds. It is known to have diverse toxic effects on cells of different visceral organs. In the present study, we purified and denatured ricin in a boiling water bath for different time intervals. We further made an attempt to identify native and denatured ricin by immunobased detection systems. All the antigen/antibody-based assays identified native and denatured ricin. On SDS-PAGE, only native ricin was observed. In western blotting, ricin boiled for 3.75 min gave a strong band on X-ray film. On native polyacryl amide gel electrophoresis, native and denatured ricin gave ricin band in 60-kDa region. The denatured ricin did cause mortality up to 25 mg/kg, while 5 and 10 µg/kg of native ricin caused 50% and 100% mortality, respectively. Detection of native and denatured ricin is very difficult, and the investigating agencies, forensic scientists, and analysts should be very careful while interpreting the results

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J Chromatogr A 2010 **1217** (5) 761

Trace determination of sulphur mustard and related compounds in water by headspace-trap gas chromatography-mass spectrometry

Through the development of a headspace-trap technique in combination with gas chromatography-mass spectrometry (GC-MS) it has become possible to detact trace levels of sulphur mustard (HD) and some of its cyclic decomposition compounds in water samples. Factorial design was employed for optimisation of the approach. Trap technology facilitates enrichment and focusing of the analytes on an adsorbent. Therefore, this method provides greater sensitivity compared with conventional static headspace. A detection limit of 1ng/ml was achieved for HD, whereas the cyclic sulphur compounds 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane could be detected at a level of 0.1ng/ml. The technique was validated for the stable cyclic compounds in the concentration range from the limit of quantification (LOQ: 0.2-0.4ng/ml) to one hundred times LOQ. The within and between assay precisions at one hundred times LOQ were 1-2% and 7-8% relative standard deviation, respectively. This approach requires almost no sample handling. The total time for sampling and analysis was less than 1h. The technique was successfully employed for both muddy river and sea water samples

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J Raman Spectrosc 2010 **41** (2) 121

Use of peptide for selective and sensitive detection of an anthrax biomarker *via* peptide recognition and surface-enhanced Raman scattering

A short 16-amino acid peptide has been used in place of an antibody to selectively detect the specific anthrax biomarker, protective antigen (PA), using surface-enhanced Raman scattering (SERS). Peptides are more stable than antibodies under various biological conditions and are easily synthesized for a specific target. A peptide that has high affinity to PA was conjugated onto gold nanoparticles along with a Raman reporter and then incubated in various concentrations of PA. Parallel studies in which the peptide sequence was replaced with an antibody were performed to compare the performance of the two methodologies. Both the peptide and antibody functionalized nanoparticles were able to specifically detect PA concentrations down to 6.1 fM. These results demonstrate that these short, robust peptides can be used in the place of traditional antibodies to specifically recognize target biomarkers in the field for the potential diagnosis of disease

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Toxicon 2010 **55** (1) 145

Silica coating magnetic nanoparticle-based silver enhancement immunoassay for rapid electrical detection of ricin toxin

Ricin toxin (RT) has been detected by employing a novel silica coated magnetic nanoparticle-based silver enhancement immunoassay (SEIA) with rapid electrical detection using interdigitated array microelectrodes (IDAMs). This novel approach was developed by employing the separation and enrichment

properties of magnetic nanoparticles (MNPs) and the catalytic properties of gold nanoparticles (GNPs). MNPs labeled with anti-ricin A chain antibody 6A6 were employed to capture ricin and GNPs labeled with anti-ricin B chain antibody 7G7 were utilised as detectors. The catalytic properties of GNPs were employed to promote silver reduction and enhance the electrical signal. In the presence of ricin, a sandwich structure was produced which could be separated by a magnetic field. The sandwich complex was then transferred to IDAMs. The silver particles bridged the IDAM gaps and produced an amplified electrical signal which was detectable by conductivity measurements. The data illustrate that the sensitivity of the SEIA with electrical detection of ricin was five times greater than that of conventional colorimetric sandwich ELISA. After the antibody employed for detection of ricin was coated onto the plates or MNPs, this new approach was three times more rapid than colorimetric sandwich ELISA. This rapid and sensitive detection procedure promises new potential for ricin detection

17 Product Authenticity

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Anal Lett 2010 **43** (4) 582

Determination of diphenylpyraline hydrochloride in pure solutions and pharmaceutical preparations using ion selective electrodes under batch and FIA conditions

New diphenylpyraline (Di) ion selective plastic membrane electrodes for both conventional and coated wire types employing the ion associate of Di-HCl with sodium tetraphenylborate (NaTPB) and phosphotungestic acid (PTA) have been fabricated. The conventional type electrode was completely determined in terms of membrane composition, plasticizer, filling solution, life span, pH, ionic strength, and temperature. Membranes of optimum composition were utilised in the production of both graphite and cupper coated wire electrodes. The electrodes were employed for the potentiometric analysis of drug content in raw material and pharmaceutical preparations under batch and flow injection analysis conditions. The selectivity of the electrodes in respect of a large number of excipients for example inorganic cations, sugars, and amino acids was examined. Furthermore, the determination of Di-HCl, the solubility product of the ion associate and the formation constant of the precipitation reaction resulting in the ion associate formation were performed employing a conductimetric method based on its ion association with NaTPB and PTA

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J Pharm Biomed Anal 2010 **51** (4) 882

A validated capillary electrophoresis method to check for batch-to-batch consistency during recombinant human glycosylated interleukin-7 production campaigns

Recombinant human glycosylated interleukin-7 (rhIL-7) batches have been produced in Chinese Hamster Ovary (CHO) cells. Quality control has been achieved following the validation of a simple CZE technique. The separation buffer was a 25mM sodium borate at pH 10 containing 12mM diaminobutane (DAB) used as a dynamic coating agent of the capillary. This procedure facilitated the separation of seven peaks ranging from low to high sialylated glycoforms. Conditioning methods of the capillary were comprehensively examined to yield repeatable results. Excellent RSD of EOF mobility (less than 0.6%) was produced when conditioning included capillary equilibration under virtual analyses and storage in 0.1M NaOH overnight. Procedure specificity was demonstrated to be capable of discriminating different rhIL-7 glycoforms produced in CHO from formulation matrix. Linearity was demonstrated between 0.5 and 4mg/ml. LOQ was 0.5mg/ml. Repeatability (RSD < 1.4 and 3.3% for t_m and A%, respectively), intermediate precision of inter-day (RSD < 2.1 and 4.5), inter-analyst (RSD < 2.0 and 3.0) and inter-equipment (RSD < 3.8 and 3.7 for electrophoretic mobility and A%, respectively) were all very satisfactory. Investigation of robustness demonstrated that pH and DAB concentration are crucial parameters in the method while slight alteration of ionic strength of electrolyte or change of capillary source did not influence the outcome. The procedure was shown to provide reliable data to interpret comparability studies and batch-to-batch consistency of biomanufactured rhIL-7

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J Liq Chromatogr Relat Technol 2010 **33** (4) 513

Simultaneous separation and determination of quinolones in pharmaceuticals by micellar liquid chromatography

The separation and determination of four quinolones (pipemidic acid, levofloxacin, norfloxacin, and moxifloxacin) in pharmaceutical preparations by a rapid and simple liquid chromatographic technique employing micellar mobile phases is described. This was accomplished in the absence of any previous pretreatment step with a C18 column employing a micellar mobile phase of 0.15 M sodium dodecyl sulphate, 2.5% propanol, and 0.5% triethylamine at pH 3, with retention times below 12 min. A diode-array UV-Vis set at 276 nm was used for detection. The limits of detection and quantification were between 8 - 51 and 28 - 171 ng/ml, respectively. The method was validated in terms of intra-day and inter-day precision and accuracy, and robustness. Calibration curves over the concentration range of 0.1 - 50 µg/ml were linear (r^2 > 0.9997). Satisfactory claim percentages (96 - 106%) were obtained in the analysis of pharmaceutical formulations. The data illustrate that the technique is appropriate for the routine analysis of drugs

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Chem Anal 2009 **54** (5) 1065

A direct spectrophotometric method for determination of antiparasitic drug nitazoxanide in a bulk form and pharmaceutical formulation

Infections caused by amitochondriate luminal parasites and helminthes may be treated with nitazoxanide, a broad-spectrum antiparasitic drug. The determination of nitazoxanide in a bulk form and pharmaceutical formulation "Cryptonaz powder" by a simple and direct UV-VIS spectrophotometric technique has been elaborated and validated. Sample pretreatment, extraction, or formation of colored chromogens prior to the analysis were not necessary. Analysis was performed in a B-R universal buffer with pH 7 containing 20% (v/v) of acetonitrile. Absorbance of nitazoxanide was measured at λ_{max} = 420 nm. Beer's law was obeyed over the nitazoxanide concentration range 1.0 x 10^{-6}-1.0 x 10^{-4} mol/l (0.293-29.30 µg/ml). Detection limit was 3.0 × 10^{-7} mol/l (0.088 µg/ml). Excipients present in "Cryptonaz powder" or degradation products of nitazoxanide produced no significant interferences. The procedure is direct, simple, precise, less time-consuming, and offers economical assay of nitazoxanide with sensitivity better than or at least equivalent with that of the reported spectrophotometric methods. Ionization constant [pk_a] ofnitazoxanide was also determined

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Anal Chem 2010 **82** (4) 1179

Direct standard-free quantitation of Tamiflu and other pharmaceutical tablets using clustering agents with electrospray ionization mass spectrometry

In order to identify counterfeit and substandard pharmaceutical drugs, accurate and rapid quantitation is important. To facilitate the direct determination of the dosage in the prescription and over-the-counter drugs Tamiflu, Sudafed, and Dramamine, a standard-free electrospray ionization mass spectrometry technique has been employed. For each drug, a tablet was dissolved in aqueous solution, filtered, and introduced into solutions containing a known concentration of l-tryptophan, l-phenylalanine, or prednisone as a clustering agent. Active ingredient(s) incorporate statistically into large clusters of the clustering agent where effects of differential ionization/detection are substantially reduced. As a result of the abundances of large clusters, the dosages of the active ingredients in each of the tablets were determined to typically better than 20% accuracy even when the ionization/detection efficiency of the individual components differed by over 100x. This technique for quantitation is unconventional and not as accurate as using conventional standards. However, it benefits are that it is fast and it may be applied to mixtures where the identities of the analytes are unknown. In addition, it may be used when suitable standards may not be readily available, for example schedule I or II controlled substances or new designer drugs which have not previously been identified

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Anal Chim Acta 2010 **658** (2) 197

Development of an immunoassay for rapid screening of vardenafil and its potential analogues in herbal products based on a group specific monoclonal antibody

Vardenafil is a phosphodiesterase-5 (PDE-5) inhibitor employed in the treatment of erectile dysfunction (ED). Undeclared vardenafil and related analogues in adulterated herbal products pose a threat to public health. An immunoassay

based on a group specific monoclonal antibody (McAb) was developed to rapidly screen for vardenafil and its analogues in a herbal matrix. Glutaraldehyde was used to link vardenafil to immunogen and coating-antigen, respectively. Following assessment of the structural specificity of eight anti-vardenafil McAbs, the McAb of 4B9 was identified as being specific to the common structure of vardenafil and its analogues. Therefore, an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) was developed based on this McAb. The limit of detection of vardenafil was 5.0 ng/ml. The calibration curve was linear from 5.0 to 40 ng/ml (R^2=0.952) with an IC_{50} value of 18.2 ng/ml. In the extracts of 20 Chinese traditional drugs, the detection capability (CCβ) of vardenafil was 0.08 mg/g, the recoveries were 76-116% and the coefficients of variation (CV%) were 9.7%-16.2%. The ic-ELISA concurred with LC-UV. The technique is a suitable tool for screening vardenafil and its analogues as illegal additives in herbal products

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J Planar Chromatogr Mod TLC 2009 **22** (6) 425

HPTLC analysis of olmesartan medoxomil and hydrochlorothiazide in combination tablet dosage forms

The analysis of olmesartan medoxomil (OM) and hydrochlorothiazide (HTZ) in combined tablet dosage forms with a new simple, accurate, and precise densitometric method is described and validated. Separation of the drugs was performed on precoated silica gel 60 F_{254} plates with chloroform-methanol-toluene 6:4:5 (v/v) as the mobile phase. Retention factors for HTZ and OM were 0.40 +/- 0.019 and 0.58 +/- 0.013, respectively. Detection of bands was performed at 258 nm. The calibration curve was linear in the concentration range 100 to 600 ng per band for OM and 50 to 300 ng per band for HTZ. For OM, the recovery data ranged from 99.92 to 100.82% with RSD values ranging from 0.300 to 0.851%. For HTZ, the recovery data ranged from 99.21 to 100.34% with RSD values ranging from 0.203 to 0.489%. The assay (%) was 99.989 +/- 0.389 and 99.516 +/- 0.303 for OM and 100.35 +/- 0.609 and 100.17 +/- 0.595 for HTZ (mean +/- S.D., n = 6), respectively in the two different tablet formulations tested. The procedure might be employed for routine analysis of these drugs in combined tablet dosage forms in quality-control laboratories

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J Pharm Biomed Anal 2010 **51** (4) 921

Analysis of crude heparin by ^{1}H NMR, capillary electrophoresis, and strong-anion-exchange-HPLC for contamination by over sulfated chondroitin sulfate

The authors have previously published details of a strong-anion-exchange-high performance liquid chromatography (SAX-HPLC) technique for the detection of the contaminant over sulfated chondroitin sulfate (OSCS) in heparin sodium active pharmaceutical ingredient (API). Whereas APIs have been treated to eliminate impurities, crude heparins contain insoluble material, chondroitin sulfates, heparan sulfate, and proteins that may interfere with the recovery and measurement of OSCS. Techniques such as 500MHz ^{1}H NMR, capillary electrophoresis (CE), and SAX-HPLC were employed to quantify OSCS in crude heparin. Using the standard API protocol on OSCS spiked crude heparin samples; a weight percent LOD and LOQ for the NMR technique of 0.1% and 0.3%, respectively were noted, whereas the SAX-HPLC procedure gave values of 0.03% and 0.09%, respectively. CE data were not amenable to quantitative measurement of OSCS in crude heparin. A modified HPLC sample preparation procedure was developed using crude heparin dissolved at the 100mg/ml level in a 2.5M NaCl solution. The SAX-HPLC technique produced a weight percent LOD of 0.02% and a LOQ of 0.07% and had better performance characteristics than that of the approach used for APIs

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Anal Chem 2010 **82** (4) 1462

Near-infrared hyperspectral unmixing based on a minimum volume criterion for fast and accurate chemometric characterization of counterfeit tablets

A crucial preliminary in the fight against pharmaceutical counterfeiting is the rapid analysis of suspect counterfeit formulations. Chemical characterization of these products will facilitate and evaluation of their impact on patient health and help regulatory authorities to identify the source. Hyperspectral unmixing of near-infrared (NIR) image data is a newly developed procedure to surmise the number of compounds, their spectral signatures, and the mixing fractions in a given tablet, with a resolution of a few tens of micrometers. In a linear mixing scheme, hyperspectral vectors belong to a simplex whose vertices correspond to the spectra of the compounds present in the sample. SISAL (simplex identification *via* split augmented Lagrangian), MVSA (minimum volume simplex analysis), and MVES (minimum-volume enclosing simplex) are recent algorithms produced to identify the vertices of the minimum volume simplex incorporating the spectral vectors and the mixing fractions at each pixel (vector). This paper illustrates the benefit of these procedures, founded on minimum volume criteria, for unmixing NIR hyperspectral data of tablets. The data indicate that SISAL/MVSA and MVES largely surpass MCR-ALS (multivariate curve resolution-alternating least-squares), which is regarded preeminent in spectral unmixing for analytical chemistry. Results are produced from synthetic data (studying the effect of noise and the presence/absence of pure pixels) and on a real data set constructed from NIR images of counterfeit tablets

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J Pharm Biomed Anal 2010 **51** (4) 991

Simultaneous HPLC analysis of pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride in liquid dosage forms

The simultaneous determination of pseudophedrine hydrochloride, codeine phosphate, and triprolidine hydrochloride in liquid formulation has been performed with an HPLC method employing UV detection. A C18 column (250mm x 4.0mm) was employed as the stationary phase with a mixture of methanol:acetate buffer:acetonitrile (85:5:10, v/v) as the mobile phase. Parameters affecting column separation of the compounds were analysed. The calibration graphs revealed a linear concentration range of 0.06-1.0 mg/ml for pseudophedrine hydrochloride, 0.02-1.0 mg/ml for codeine phosphate, and 0.0025-1.0 mg/ml for triprolidine hydrochloride for a sample size of 5µl with correlation coefficients of better than 0.999 for all active ingredients investigated. The results provide evidence that this technique is reliable, reproducible and applicable for routine use with an analysis time of less than 4min

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J Pharm Biomed Anal 2010 **51** (4) 994

Determination of duloxetine hydrochloride in the presence of process and degradation impurities by a validated stability-indicating RP-LC method

A purity and assay technique for duloxetine hydrochloride (DUH) has been developed and validated employing stability-indicating gradient reverse phase liquid chromatography. DUH was exposed to the stress conditions and proved sensitive in respect of oxidative, acid and hydrolytic degradation. DUH from its two process impurities and one degradation impurity formed under stress conditions were successfully separated on a Symmetry C18, 250 x 4.6mm, 5µm column using a gradient mixture of solvent A (0.01M potassium dihydrogen orthophosphate having 0.2% triethyl amine, pH adjusted to 2.5 with orthophosphoric acid) and solvent B (20:80 v/v mixture of acetonitrile and methanol). The flow rate was 1ml/min and the detection wavelength was 230nm. The mass balance was noted to be in the range of 99.2-99.7% in all the stressed conditions

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J Liq Chromatogr Relat Technol 2010 **33** (3) 413

A validated HPLC method for separation and determination of epinastine hydrochloride enantiomers

The separation and determination of epinastine hydrochloride by a simple, rapid, and validated technique has been developed and is described. Epinastine hydrochloride enantiomers were separated and determined on a Chiralcel® OD-R column (250 4.6 mm i.d., 0.5 µm particle size), using a mixture of *n*-hexane: isopropanol: diethylamine: triflouroacetic acid (85: 15: 0.1: 0.1% v/v/v/v) as a mobile phase at 20 °C and at a flow rate of 1 ml/min. The UV detector was set to 254 nm. Epinastine hydrochloride 1000 µg/ml was employed as an external standard. The HPLC procedure facilitated the separation and quantification of epinastine hydrochloride enantiomers with good linearity (r > 0.999) in the studied range. The relative standard deviations (RSD) were 1.076 and 0.769% for the epinastine hydrochloride enantiomers with accuracy of 99.65 and 99.77 for the enantiomeric pair separated. The limit of detection and limit of quantification of epinastine hydrochloride enantiomers were found to be 20 and 60 µg/ml, respectively. The technique was validated through the parameters of linearity, accuracy, precision, and robustness. The above technique was employed for the quantitative determination of epinastine hydrochloride in pharmaceutical formulations

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J Pharm Biomed Anal 2010 **51** (5) 1037

Identification and characterization of degradation products of irbesartan using LC-MS/TOF, MS^n, in-line H/D exchange and LC-NMR

In accordance with ICH guideline Q1A (R2), irbesartan was subjected to hydrolytic, oxidative, photolytic and thermal stress. Irbesartan exhibited degradation only in acidic, basic and photoacidic conditions but was stable to other forms of stress. Three degradation compounds were produced and these were separated on a C-8 column using a gradient HPLC method. A complete mass fragmentation pathway of the drug was derived by means of MS/TOF, MS^n and H/D exchange studies. Subsequently, the degradation products were analysed with LC-MS/TOF and on-line H/D exchange mass studies to reveal accurate mass, fragment pattern and number of labile hydrogens. The MS results provided tentative structures of degradation products and these were verified by ^{1}H and 2D COSY LC-NMR. The products were identified as (2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methanamine, 1-(1-((2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methylamino)pentylideneamino)cyclopentane carboxylic acid and 2-butyl-3-(tetrazolo[1,5-*f*]phenanthridin-6-ylmethyl)-1,3-diazaspiro[4.4]non-1-en-4-one. Structures were rationalised by mechanisms of formation

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J Chromatogr Sci 2010 **48** (2) 109

Stability-indicating TLC-densitometric determination of nebivolol hydrochloride in bulk and pharmaceutical dosage form

In accordance with the International Conference on Harmonization guidelines (ICH), a simple, selective, precise, and stability-indicating high-performance thin-layer chromatographic (HPTLC) procedure was developed and validated for densitometric determination of nebivolol hydrochloride both as a bulk drug and in formulations. The procedure utilises TLC aluminium plates precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system incorporates toluene-methanol-triethylamine (3.8:1.2:0.2 v/v/v). Densitometry analysis of nebivolol hydrochloride was performed in the absorbance mode at 281 nm. The system was noted to provide a discreet spot for nebivolol hydrochloride (R_f value of 0.33 +/- 0.02). The linear regression analysis data for the calibration plots showed good relationship with r^2= 0.9994 +/- 0.0002 in the concentration range 500-3000 ng/spot. The mean value +/- SD of slope and intercept were 3.761 +/- 0.017 and 127.39 +/- 19.53 in respect of peak area. The limits of detection (LOD) and limit of quantitation (LOQ) were 63.10 ng/spot and 191.23 ng/ spot, respectively. Nebivolol hydrochloride was subjected to acid and alkali hydrolysis, oxidation, thermal degradation, and photodegradation. All the peaks of degradation products were well-resolved from the standard drug with significantly different R_f values. Statistical analysis demonstrates that the technique is repeatable, selective and accurate. The HPTLC technique may be employed for identification and quantitative determination of nebivolol hydrochloride in the bulk and pharmaceutical dosage form

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J Planar Chromatogr Mod TLC 2009 **22** (6) 411

Stress degradation studies on valsartan using validated stability-indicating high-performance thin-layer chromatography

The angiotensin-II AT_1-receptor is a target for the treatment of hypertension. Valsartan, administered orally, is a potent and specific competitive antagonist. A sensitive, selective, precise, and stability-indicating high-performance thin-layer chromatographic procedure for analysis of valsartan both as the bulk drug and in formulations has been developed and validated. The technique employs aluminum-backed silica gel $60F_{254}$ plates with toluene-ethyl acetate-methanol-formic acid 60.0:20.0:20.0:1.0 (v/v) as mobile phase. The protocol produced compact bands for valsartan (R_F 0.44 +/- 0.05). Densitometric analysis of valsartan was achieved with absorbance mode at 250 nm. Linear regression analysis data for the calibration plots demonstrated a good linear relationship with r^2 = 0.9946 +/- 0.0013 in the working concentration range 200 to 1600 ng per band. The procedure was validated for precision, robustness, and recovery. Limits of detection and quantification were 25 and 150 ng per band, respectively. Valsartan was subjected to different stress conditions as prescribed by International Conference on Harmonization guidelines. These included acid and alkaline hydrolysis, oxidation, photo degradation, and dry and wet heat treatment. Degradation products were well differentiated from the pure drug with significantly different R_F values. This procedure might effectively separate the drug from its degradation products. Therefore, it might be used as a stability-indicating technique for the analysis of valsartan

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J Planar Chromatogr Mod TLC 2009 **22** (6) 399

Densitometric analysis of celecoxib, etoricoxib and valdecoxib in pharmaceutical preparations

The analysis of celecoxib, etoricoxib, and valdecoxib in pharmaceutical formulationswith a densitometric thin-layer chromatographic procedure is described. TLC was accomplished on silica gel 60 F_{254} plates using chloroform-acetone-toluene 12:5:2 (v/v) as mobile phase. UV detection was achieved by densitometric scanning at 254 and 290 nm. The procedure was validated by determination of linearity, precision, limits of detection, and determination (from 0.0017 to 0.0848 μg per band) and accuracy (from 99.16 to 100.48%).

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J Pharm Biomed Anal 2010 **51** (4) 858

Structural elucidation of the Rifaximin Ph. Eur. Impurity H

Rifaximin is a semisynthetic, rifamycin-based non-systemic antibiotic employed in the treatment of acute and chronic gastrointestinal disorders. The purpose of this research was to determine the molecular structure of the 802 Dalton impurity which was found in Rifaximin industrial batches and reported with an erroneous structure in European Pharmacopoeia 6.5 (2009) [7] monograph as Rifaximin Impurity H. This impurity was isolated from Rifaximin with preparative HPLC and purified with column chromatography. The molecular structure was determined using ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and FT-IR

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Rapid Commun Mass Spectrom 2010 **24** (11) 1697

A gas chromatography/mass spectrometry method for the determination of sildenafil, vardenafil and tadalafil and their metabolites in human urine

Sildenafil (SDF), vardenafil (VDF) and tadalafil (TDF) are phosphodiesterase type 5 enzyme inhibitors (PDE5Is), used in the treatment of erectile disorders and to improve breathing efficiency in pulmonary hypertension. The increasing incidence of their use among young athletes has drawn the attention of the anti-doping authorities to the possible abuse of PDE5Is by athletes due to their pharmacological activities. This paper describes a method for the determination in urine of PDE5Is and their metabolites by gas chromatography/mass spectrometry (GC/MS) after liquid/liquid extraction of the analytes from urine and derivatisation to obtain trimethylsilyl derivatives. The metabolic profile was studied on real samples collected from subjects taking PDE5Is (Viagra, Levitra or Cialis); the main urinary metabolites were identified and their MS fragmentation characterized. The sample pre-treatment and GC/MS conditions for the detection of the metabolites have been optimised. A method for their preliminary screening and subsequent confirmation is described that takes into account the general requirements of a routine doping analysis to be used for the screening of large numbers of samples. The main metabolites identified can be included in a general purpose screening method and all the metabolites in a more specific confirmation method. The method developed has been applied for the screening of PDE5Is in 5000 urine samples. Based on the obtained results, the proposed method appears to be of practical use in analytical and forensic toxicology, including doping analysis

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J Pharm Biomed Anal 2010 **51** (5) 1060

Determination of potency of heparin active pharmaceutical ingredient by near infrared reflectance spectroscopy

A crucial aspect in analysis of quality of heparin active pharmaceutical ingredient (API) is potency. The potency of heparin API was determined with near infrared reflectance spectroscopy (NIRS) coupled with partial least squares (PLS) algorithm. PLS factors, correlation coefficient of calibration set (R_c), the root mean square of cross-validation (RMSECV), correlation coefficient of prediction set (R_p) and the root mean square of prediction (RMSEP) were employed to appraise the performance of the models. The optimal calibration model was obtained with R_p = 0.9721 and RMSEP = 0.55 in the 1700-1898nm spectral region when using SG-1st derivative spectral transform method and division of calibration/prediction samples was 1/1. Three further samples showed good prediction capability of the final model and three validation samples produced good result repeatability. NIRS has the capability to be a final lot release test when performed in a QC laboratory

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Anal Sci 2010 **26** (1) 121

Quantitative spot-test analysis of metformin in pharmaceutical preparations using ultraviolet-visible diffuse reflectance spectroscopy

The analysis of metformin in pharmaceutical preparations with a simple, quantitative spot-test using diffuse UV-visible reflectance is described. The method relies upon the formation of a metformin-nickel(II) complex on a glass filter membrane followed by measurement of the reflectance with a spectrophotometer employing an integration sphere. Data produced with commercial products were compared statistically with those produced by employing a protocol recommended by JP and by USP. Complete agreement was noted. The average RSD was 2.5%. The detection (0.009 mol/l) and the quantitation (0.03 mol/l) limits are quite acceptable for pharmaceutical analysis

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J Planar Chromatogr Mod TLC 2009 **22** (6) 421

A simple and sensitive HPTLC method for simultaneous analysis of domperidone and paracetamol in tablet dosage forms

The simultaneous analysis of domperidone (DMP) and paracetamol (PAR) in tablet dosage forms has been achieved with a simple, precise, rapid, selective, and economic high-performance thin-layer chromatographic (HPTLC) technique. The chromatographic separation was accomplished on precoated silica gel 60 GF_{254} plates with acetone-toluene-methanol 4:4:2 (v/v) as mobile phase. Plates were developed to a distance of 8.0 cm at ambient temperature. Developed plates were scanned and quantified at their single wavelength of maximum absorption of approximately 285 and 248 nm for domperidone and paracetamol, respectively. Experimental conditions were optimized following analysis of band size, chamber saturation time, migration of solvent front, slit width, etc. Both drugs were satisfactorily resolved with R_F 0.52 +/- 0.02 for domperidone and 0.74 +/- 0.02 for paracetamol. The technique was validated for linearity, accuracy, precision, and specificity. The calibration plot was linear between 16–48 ng per band for DMP and 800–2400 ng per band for PAR. Limits of detection and quantification for DMP were 0.022 and 0.186 ng per band, respectively. For PAR they were 0.307 and 0.931. The HPTLC technique described is economic, sensitive, and less time consuming than other chromatographic procedures

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Anal Lett 2010 **43** (3) 373

Rapid resolution RP-HPLC-DAD method for simultaneous determination of sildenafil vardenafil and tadalafil in pharmaceutical preparations and counterfeit drugs

The simultaneous determination of sildenafil, vardenafil, and tadalafil in pharmaceutical preparations and counterfeit drugs has been achieved following the development and validation of a novel and rapid resolution reversed-phase high-performance liquid chromatography-diode array detector technique. An Agilent Zorbax SB C8 column (50 4.6 mm i.d., 1.8 µm particle size) was employed. The mobile phase comprised a mixture of 0.030 M ammonium formate (adjusted to pH 3.0 with formic acid) and acetonitrile in the ratio 70:30. Ultraviolet (UV) detection was performed at 230 nm. Total run time was 7 min. The three drugs were eluted with retention times of 1.654, 2.032, and 5.067 min for vardenafil, sildenafil, and tadalafil, respectively. The procedure was validated in respect of accuracy, precision, linearity, specificity, and sensitivity. From the validation study, it was noted that the technique was specific, rapid, accurate, precise, and reproducible. Calibration curves were linear over the concentration ranges of 0.2-200 µg/ml for sildenafil, vardenafil, and tadalafil. Limits of detection (LOD) values were 1.0, 1.1, and 1.0 ng and the limit of quantification (LOQ) values were 2.0, 2.1, and 2.0 ng for sildenafil, vardenafil, and tadalafil, respectively. The procedure is rapid both for routine quantitative analysis of sildenafil, vardenafil, and tadalafil in pharmaceutical preparations and for screening counterfeits

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J Liq Chromatogr Relat Technol 2010 **33** (4) 452

A simple and convenient method for simultaneous determination of four major species of illegal additives in slimming health food

Four major species of illegal additives (orlistat, cetilistat, sibutramine, and rimonabant) in health food have been simultaneously determined by the development of a simple and convenient approach. Analytes were separated and quantified with HPLC-PDA at 222 nm. Analytical separation was achieved by employing gradient elution with acetonitrile and 0.02 mol/l phosphoric acid aqueous solution on a C_{18} column. Recovery was in the range of 96.1 - 97.2%. The procedure was reproducible with intra- and inter-day variation of less than 1%. The limit of detection was 189 ng/ml and the calibration curves showed good linearity ($R^2 > 0.999$). When compared with previous methods, the proposed technique has the benefits of simplicity, rapidity, and good applicability. It was applied successfully to analyze market products

18 Techniques

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J Chromatogr A 2010 **1217** (3) 302

Enhancing the detection sensitivity of trace analysis of pharmaceutical genotoxic impurities by chemical derivatization and coordination ion spray-mass spectrometry

Genotoxic impurities in many pharmaceuticals are neutral molecules. Analysis of traces of these neutral analytes is hinderered by their poor ionization efficiency in mass spectrometry (MS). Two analytical protocols including chemical derivatization and coordination ion spray-MS have been produced to enhance neutral analyte detection sensitivity. Chemical derivatization converts analytes into highly ionizable or permanently charged derivatives, which become readily detectable by MS. The coordination ion spray-MS method enhances ionization by forming neutral-ion adducts with metal ions such as Na^+, K^+, or NH_4^+ which are introduced into the electrospray ionization source. Both procedures have been demonstrated to improve the detection sensitivity of neutral pharmaceuticals substantially. In this paper, the successful applications of the two approaches is demonstrated for the analysis of four pharmaceutical genotoxic impurities identified in a single drug development program. Two were non-volatile alkyl chlorides and the other two epoxides

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J Planar Chromatogr Mod TLC 2009 **22** (6) 435

Development of conditions for rapid thin-layer chromatography of -lactam antibiotics

Penicillins and cephalosporins (subclasses of β-lactam antibiotics) are routinely employed against Gram-positive and Gram-negative bacteria. HPLC, TLC-bioautography, and thin silica gel layers precoated with fluorescent material have been reported in literature for the analysis of these compounds. This paper describes a straightforward and sensitive method for rapid separation and detection of selected β-lactams. Bulk impregnation of homemade silica gel G layers and impregnation of ready made silica gel 60 layers with 0.2% ammonium chloride was performed and various mobile phases were established for UV detection of the compounds. Separation of penicillins (benzylpenicillin, ampicillin, and amoxicillin) and cephalosporins (cephalexin, cefoperazone, ceftriaxone, cefixime, and cefadroxil) was accomplished with propanol-acetic acid 4:1 (v/v) and butanol-acetic acid-water 4:1:2 (v/v) respectively, as mobile phases

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J Planar Chromatogr Mod TLC 2009 **22** (6) 417

HPTLC method for simultaneous analysis of escitalopram oxalate and clonazepam in pharmaceutical preparations

The simultaneous analysis of escitalopram oxalate (ESC) and clonazepam (CLO) in pharmaceutical preparations has been achieved by the development of a simple, rapid, precise, and accurate high-performance thin-layer chromatographic (HPTLC) technique which had subsequently been validated. Separation was accomplished on aluminum HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} with methanol-toluene-triethylamine 1:3.5:0.1 (v/v) as mobile phase. Densitometric quantification was carried out at 253 nm by reflectance scanning. The R_F values of ESC and CLO were 0.36 and 0.49, respectively. The linearity of the technique was investigated in the range 50–150 µg/ml for ESC and 5–15 µg/ml for CLO. Recovery percentages for ESC and CLO were 100.41 and 99.54%, respectively, by height and 100.21% and 99.99% by area. The technique was validated in terms of precision, accuracy, specificity, and ruggedness

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J Planar Chromatogr Mod TLC 2009 **22** (6) 405

Simultaneous analysis of hydrochlorothiazide, triamterene, furosemide, and spironolactone by densitometric TLC

Hydrochlorothiazide, triamterene, furosemide, and spironolactone may be jointly present in complex drugs employed to treat hypertension. A new chromatographic and densitometric is described for their identification and quantitative analysis. Separation was achieved with silica gel F_{254} plates employing hexane-ethyl acetate-methanol-water-acetic acid 8.4:8:3:0.4:0.2 (v/v) as the mobile phase. Densitometric measurements were carried out at 264 nm selected for all of the constituents. The procedure is specific for the analyte constituents examined, and characterized by high sensitivity. LOD was 0.022 to 0.150 µg per band. LOQ was from 0.068 to 0.450 µg per band. Recovery was from 97.10 to 101.02%. Linear range was 0.060 to 2.650 µg per band. The technique was characterized by good precision with RSD from 0.66 to 0.96%

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High-performance liquid chromatographic method for the separation of enantiomeric gatifloxacin

The separation of enantiomeric gatifloxacin, (+/-) 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid, an antibiotic in bulk drug has been accomplished by the development of a high-performance liquid chromatographic procedure. The technique utilises an amylose-based Chiralpak AD-H (150 mm x 4.6 mm, 5 µm) column employing a mobile phase system of *n*-hexane-ethanol-diethylamine (85:15:0.1% v/v). Conditions were optimised by selection and variation of the mobile-phase compositions. Differences in separation capability of the technique were also noted. Relative standard deviation of retention times and peak areas were better than 0.2% and 0.4%, respectively, for precision. Gatifloxacin sample solution and mobile phase were found to be stable for at least 48 h

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Rapid and highly sensitive HPLC and TLC methods for quantitation of amlodipine besilate and valsartan in bulk powder and in pharmaceutical dosage forms and in human plasma

The simultaneous estimation of amlodipine besilate (AM) and valsartan (VL) has been achieved by the development of two simple, sensitive, and specific high-performance liquid chromatography and thin-layer chromatography techniques. Separation by HPLC was accomplished by employing a xTerra C18 column and methanol/acetonitrile/water/0.05% triethylamine in a ratio 40:20:30:10 by volume as mobile phase, pH was adjusted to 3 +/- 0.1 with *o*-phosphoric acid. The flow rate was 1.2 ml/min. The linearity range was 0.2 to 2 µg/ml for amlodipine besilate and 0.4 to 4 µg/ml for valsartan with a mean percentage recovery of 99.59 +/- 0.523% and 100.61 +/- 0.400% for amlodipine besilate and valsartan, respectively. The TLC method utilised silica gel 60 F_{254} plates and the optimized mobile phase was ethyl acetate/ methanol/ ammonium hydroxide (55:45:5 by volume). Quantitatively, the spots were scanned densitometrically at 237 nm. The range was 0.5-4.0 µg/spot for amlodipine besilate and 2.0-12.0 µg/spot for valsartan. The mean recovery was 99.80 +/- 0.451% and 100.61 +/- 0.363% for amlodipine besilate and valsartan, respectively. The HPLC technique was found to be simple, selective, precise, and reproducible for the measurement of both drugs in spiked human plasma

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Enantiomeric separation of *S*-zopicione and its *R*-enantiomer in bulk drug samples by validated chiral RP-HPLC

The enantiomeric purity determination of *S*-zopiclone and the quantitative determination of *R*-zopiclone in bulk drug samples has been achieved by development of a simple and selective isocratic chiral RP-HPLC technique. Enantiomeric separation was performed on a Chiralcel OD-RH 150 x 4.6 mm, 5 µm particle size column at 25 °C using a mobile phase of 10 mM ammonium acetate and acetonitrile in ratio of 60:40 (v/v) as mobile phase at a flow rate of 1.0 ml/min and UV detection at 306 nm. The procedure resolves the *R*-zopiclone and *S*-zopiclone with resolution (Rs) greater than 1.6. The limit of detection (LOD) and limit of quantitation (LOQ) of the *R*-enantiomer were 0.12 µg/ml and 0.40 µg/ml respectively, for 10 µl injection volume. The percentage RSD of the peak area of six replicate injections of *R*-zopiclone at LOQ concentration was 4.6. The percentage recoveries of *R*-enantiomer from *S*-zopiclone were ranged from 97.3 to 99.8. The procedure was found to be selective in presence potential impurities and has been validated in respect of precision, linearity, accuracy, robustness and ruggedness. The test solution and mobile phase were found to be stable up to 24 h after preparation

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Development and validation of a stability-indicating HPLC-UV method for the determination of alizapride and its degradation products

The determination of alizapride (AL) and its main degradation products alizapride carboxylic acid (AL-CA) and alizapride *N*-oxide (AL-NO2) in drug substance and product has been achieved by the development of a stability-indicating high-performance liquid chromatography technique. The procedure was based on forced degradation data obtained by HPLC-MS analysis. AL was chemically degradaded by acid/base catalyzed hydrolysis and oxidation. The main degradation products were AL-CA and AL-NO2. Separation and quantification were accomplished on a 150-mm reverse phase column with a hydrophilic linkage between silica particles and hydrophobic alkyl chains. The mobile phase (flow rate 1.5ml/min) was comprised of eluant A [aqueous acetate buffer (pH 4.0; 20mM)] and eluant B [CH_3OH] with gradient elution and detection of analytes at 225nm. The technique displayed good linearity for the AL, AL-CA, AL-NO2 mixture in the 25-75, 1-15 and 1-15 µg/ml ranges respectively [r^2 greater than 0.999]. Intra-day and inter-day precisions and expressed as RSDs were 0.8, 1.3 and 2.1% and 1.0, 1.7, 4.8% for AL, AL-CA and AL-NO2 respectively. Average recoveries, at 100% and 0.2% of the target assay concentration, were 100.5, 98.6, and 96.8% for AL, AL-CA and AL-NO2 respectively. Robustness was also examined by variations of mobile phase composition and pH. The applicability of the method was investigated in commercial dosage form analysis and in stability studies

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LC/MS characterization of impurities and degradation products of a potent antitumor peptidic dimer, CU201

Compound CU201 [SUIM-(D-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-d-Igl-Oic-Arg)$_2$, where SUIM=suberimidyl; Hyp=*trans*-4-hydroxyproline; Igl=α-(2-indanyl)-glycine; Oic=octahydroindole-2-carboxylic acid], is a dimeric analog of the potent bradykinin antagonist peptide B9430. It blocks the $G_{\alpha q,11}$ signal of the heterotrimeric G proteins, stimulates c-Jun kinases, and induces apoptosis in lung cancer cells with neuroendocrine features. CU201 exhibits potent inhibition of small-cell lung cancer cells *in vitro* (ED_{50} = 0.15µM) and of small-cell lung cancer SHP-77 tumor growth *in vivo*. An HPLC technique has been developed, as part of research supported by the National Cancer Institute's (NCI's) Rapid Access to Interventional Development (RAID) program, to assess the purity and stability of CU201. Impurities and degradation products were analyzed with LC/MS. The identity of a major impurity, with 1 mass unit different from CU201, was confirmed by high resolution LC/MS and the investigation of model compounds. Susceptible linkages in the peptide chains were demonstrated by a degradation study

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Simultaneous determination of ezetimibe and simvastatin in pharmaceutical preparations by MEKC

The simultaneous determination of ezetimibe and simvastatin in pharmaceutical preparationshas been achieved by the development and validation of a micellar electrokinetic capillary chromatography technique. The influence of buffer concentration, buffer pH, sodium dodecyl sulphate (SDS) concentration, organic modifier, capillary temperature, applied voltage, and injection time was examined and validation studies were performed. Optimum separation for the compounds was accomplished in less than 10 min at 30 °C with a fused-silica capillary column (56 cm x 50 µm i.d.) and a 25mM borate buffer at pH 9.0 containing 25mM SDS and 10% (v/v) acetonitrile. Samples were injected hydrodynamically for 3 s at 50 mbar, and the applied voltage was +30.0 kV. Detection wavelength was set at 238 nm and diflunisal was used as internal standard. The technique was adequately validated in respect of stability, specificity, linearity, limits of detection and quantification, accuracy, precision, and robustness. LODs and LOQs were 1.0 and 2.0 µg/ml for ezetimibe and simvastatin, respectively. The method was successfully used in the simultaneous determination of ezetimibe and simvastatin in pharmaceutical preparations