Published online in Wiley InterScience 2010

(www.interscience.wiley.com) DOI:10.1002/dta.63

Current awareness in drug testing and analysis

1 Reviews

Bhushan R, Kumar R// Indian Inst Technol, Dept Chem, IN-247667 Roorkee, India
Biomed Chromatogr 2010 **24** (1) 66
Enantioresolution of *dl*-penicillamine
Penicillamine (PenA) is a nonproteinogenic amino acid containing a thiol group. The three functional groups in penicillamine undergo characteristic chemical reactions and differ in their ability to participate in various chemical and biochemical reactions. D-penicillamine is more active pharmacologically, while the L-isomer occurs 'naturally'. This review deals with the enantioresolution of PenA both by direct and indirect methods using liquid chromatography. HPLC separation of its diastereomers prepared with different chiral derivatizing reagents (on reversed-phase columns) and separation of the derivatives prepared with achiral reagents (on chiral columns or *via* ligand exchange mode) has been discussed. Separation of enantiomers tagged with achiral reagent (to add a chromophore for ehanced detection) when there is no diastereomer formation has been considered separately. In addition, application of PenA and its derivatives as chiral selector for enentioresolution of certain other compounds has also been discussed

Daeid NN, Buchanan HAS, Savage KA, Fraser JG, Cresswell SL// Univ Strathclyde, Dept Pure & Appl Chem, Ctr Forensic Sci, Glasgow G1 1XW, Scotland
Aust J Chem 2010 **63** (1) 3
Recent advances in the application of stable isotope ratio analysis in forensic chemistry
This review paper updates the previous literature in respect of the continued and developing use of stable isotope ratio analysis in samples which are relevant to forensic science. Recent advances in the analysis of drug samples, explosive materials, and specimens obtained from human and animal samples are discribed. The paper also addresses the use of isotope ratio mass spectrometry in a forensic context and discuss its evidential potential

Kataoka H// Shujitsu Univ, Sch Pharm, Okayama 703 8516, Japan
Anal Bioanal Chem 2010 **396** (1) 339
Recent developments and applications of microextraction techniques in drug analysis
Sample preparation is crucial for isolating desired components from complex matrices and greatly afects their reliable and accurate analysis. Recent developments in sample preparation include miniaturization, automation, high-throughput performance, online coupling with analytical instruments, and low-cost operation through extremely low or no solvent consumption. Microextraction procedures, for example liquid-phase microextraction and solid-phase microextraction, incorporate these advantages when compared with the traditional approaches of liquid-liquid extraction and conventional solid-phase extraction. This review highlights microextraction techniques produced over the last decade and presents a synopsis of the attributes of various approaches in drug analysis

Llaquet H, Pichini S, Joya X, Papaseit E, Vall O, Klein J, Garcia-Algar O*// *Hosp Mar, IMIM, Unitat Recerca Infancia & Entorn, Pg Maritim 25-29, ES-08003 Barcelona, Spain
Anal Bioanal Chem 2010 **396** (1) 379
Biological matrices for the evaluation of exposure to environmental tobacco smoke during prenatal life and childhood
Biomarker assessment of the exposure to environmental tobacco smoke during childhood has employed the measurement of nicotine and its major metabolites cotinine and *trans*-3 -hydroxicotinine together with other minor metabolites (e.g., cotinine *N*-oxide, cotinine, and *trans*-3 -hydroxicotinine glucuronides) in conventional and nonconventional biological matrices. Determination of these compounds in matrices such as amniotic fluid, meconium, and fetal hair demonstrates prenatal exposure to cigarette smoking at different stages of pregnancy. Nicotine and its metabolites in cord blood, neonatal urine, and breast milk facilitate analysis of acute exposure to drugs of abuse in the period immediately before and after delivery. Cotinine measurement in children's blood and urine and nicotine and cotinine measurements in children's hair provide assessment of acute and chronic exposure during infancy, respectively. However, for appraisal of cumulative exposure to environmental tobacco smoke during the entire childhood, including the prenatal period, analysis of nicotine in teeth has been suggetsed as a promising noninvasive tool. This article reviews the usefulness of measurement of nicotine and its metabolites in different fetal and pediatric biological matrices in respect of noninvasive collection, time window of exposure detection and finally clinical application in pediatrics

Rupert JL// Univ British Columbia, Sch Human Kinetics, 6081 University Blvd, Vancouver, Brit Columbia, Canada V6T 1Z1
Scand J Med Sci Sports 2009 **19** (6) 753
Transcriptional profiling: A potential anti-doping strategy
Evolving challenges require evolving responses. The use of illicit performance enhancing drugs by athletes permeates the reality and the perception of elite sports. New drugs with ergogenic or masking potential are quickly adopted, driven by a desire to win and the necessity of avoiding detection. To counter this trend, anti-doping authorities are continually refining existing assays and developing new testing strategies. In the post-genome era, genetic- and

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.

molecular-based tests are being evaluated as potential approaches to detect new and sophisticated forms of doping. Transcriptome analysis, in which a tissue's complement of mRNA transcripts is characterized, is one such method. The quantity and composition of a tissue's transcriptome is highly reflective of milieu and metabolic activity. There is much interest in transcriptional profiling in medical diagnostics and, as transcriptional information can be obtained from a variety of easily accessed tissues, similar approaches could be used in doping control. This article briefly reviews current understanding of the transcriptome, common methods of global analysis of gene expression and non-invasive sample sources. While the focus of this article is on anti-doping, the principles and methodology described could be applied to any research in which non-invasive, yet biologically informative sampling is desired

Scott KS// Univ Glasgow, Glasgow G12 8QQ, Scotland
Sci Justice 2009 **49** (4) 250
The use of hair as a toxicological tool in DFC casework
Frequently, when drug-related offences are cited, it is generally assumed to be sexual assault. However, the law includes any crime committed whilst the complainant is affected by alcohol or drugs, i.e., the use of a drug to influence a person's behaviour for criminal gain. Cases include robbery, blackmail and of course sexual offences. Hair analysis for drugs is now routine in forensic toxicology. Its deployment as an analytical tool in workplace testing, post-mortem toxicology and criminal cases is incresing both in the U.K. and worldwide. It is now widely accepted as an alternative or complimentary specimen in these cases. This paper provides a brief overview of hair analysis in cases of drug-facilitated crime emphasising the importance of timely specimen collection. Its pertinence to cases of this type is spotlighted by case examples

Tagliaro F, Pascali J, Fanigliulo A, Bortolotti F// Univ Verona, Dept Med & Publ Hlth, Sect Forensic Med, Policlin GB Rossi, Ple LA Scuro, IT-37134 Verona, Italy
Electrophoresis 2010 **31** (1) 251
Recent advances in the application of CE to forensic sciences: A update over years 2007-2009
The purpose of this review is to focus on the applications of CE to forensic sciences covering the short period from 2007 until the first months of 2009. This is an update of two previous review papers covering the years from 2001 to 2004 and from 2005 to 2007. The overview includes the most relevant examples of analytical applications of capillary electrophoretic and electrokinetic techniques in a number of fields. (i) Illicit and abused drugs. (ii) Small ions of forensic interest (iii) Proteins and peptides. (iv) Forensic deoxyribonucleic acid. (v) Dyes and inks

Thevis M, Hemmersbach P, Geyer H, Schanzer W// Deutsche Sporthochschule Koln, Inst Biochem, Zentrum Praventive Dopingforschung, Am Sportpark Mungersdorf 6, DE-50933 Cologne, Germany
Med Klin 2009 **104** (12) 918
Doping in disabled sports. Doping control activities at the Paralympic Games 1984-2008 and in Germany 1992-2008 (German, English Abstract)
Endeavours in respect of the fight against doping with regard to the Paralympic Games commenced in 1984, when the first doping controls were performed. Exactly 20 years ago (1989), the foundation of the International Paralympic Committee substantially endorsed systematic sports drug-testing programs specifically designed to meet the particular challenges related to disabled sports. This produced a variety of unfavourable analytical findings (e.g., with anabolic steroids, diuretics, corticosteroids, and stimulants) particularly at Paralympic Summer Games. In Germany, doping controls for disabled athletes were established in 1992 and have been performed since by the National Paralympic Committee Germany and the National Anti-Doping Agency. Various similarities in terms of antidoping rule violations were discovered when compared with doping controls of nondisabled athletes. In this paper, a summary is presented of analyses of specimens from the Paralympic Summer and Winter Games in respect of the doping control program for disabled sports in Germany. Details of sample collection and the doping method termed "boosting" are presented

2 Sports Doping - General

Murray GI, Danaceau JP// Univ Utah, Ctr Human Toxicol, 417 Wakara Way, Salt Lake City, Ut 84112, USA
J Chromatogr B 2009 **877** (30) 3857
Simultaneous extraction and screening of diuretics, -blockers, selected stimulants and steroids in human urine by HPLC-MS/MS and UPLC-MS/MS
High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) and ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) protocols have been employed for the simultaneous determination of 49 exogenous compounds (21 diuretics, 19 β-blockers, eight stimulants and two steroidal drugs) in human urine. Urine samples weretreated with a simple and robust solid phase extraction (SPE) method. Samples were injected onto reversed phase HPLC and UPLC columns connected to tandem mass spectrometers capable of scan-to-scan polarity switching. The procedures were validated according to the ISO 17025 international standard for the validation of a qualitative method. Sixty urine samples submitted for routine analysis were tested with both techniques. Data correlate with results obtained from previously validated procedures. Both procedues facilitated routine urine analysis and in paricular the use of UPLC-MS/MS demonstrated that samples may be reliably screened with significantly reduced analysis times

3 Steroids

Mazzarino M, Bragano MC, Donati F, De la Torre X, Botre F*// *Antidoping Lab Rome, Largo Giulio Onesti 1, IT-00197 Rome, Italy
Anal Chim Acta 2010 **657** (1) 60
Effects of propyphenazone and other non-steroidal anti-inflammatory agents on the synthetic and endogenous androgenic anabolic steroids urinary excretion and/or instrumental detection
Gas-chromatography mass-spectrometry was employed to assess the effects of oral administration of non-steroidal anti-inflammatory drugs on urinary excretion of endogenous and synthetic anabolic androgenic steroids. Analyses were performed on specimens from 5 male subjects with pathologies and/or diseases, treated with non-steroidal anti-inflammatory drugs. To establish the individual baseline variability of testosterone and its main metabolites, urine samples were collected for 3 days, every 2 h prior to the administration of the drug(s). To study of the effects of a single dose of each drug on the endogenous androgen steroid urinary concentrations urine samples was collected for 2 days, every 2 h. Data obtained after drugs administration were then analysed whilst considering the individual baseline variability. Only propyphenazone administration resulted in the relative urinary concentrations of some testosterone metabolites being significantly altered. Urinalysis demonstrated that levels of dehydroepiandrosterone, 11keto-etiocholanolone, 11β-hydroxyandrosterone, 11β-hydroxyetiocholanolone, androsterone, etiocholanolone and some metabolite ratios decreased significantly, generally between 2 and 10 h after administration of the drug. However, no effects were noted calculated concentrations of testosterone, epitestosterone, 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol and testosterone/epitestosterone ratio in urine. The observed effects do not depend on alterations on pharmacokinetics (excretion/metabolism), but rather steroid sample preparation steps (hydrolysis and derivatization) inhibition. The significant decrease of dehydroepiandrosterone and testosterone metabolites urinary levels resulted from a reduced yield in the steroid derivatization step for the presence of the main metabolites of propyphenazone, namely hydroxyl-propyphenazone metabolites

Mitrevski BS, Wilairat P, Marriott PJ*// *RMIT Univ, Sch Appl Sci, Australian Ctr Res Separation Sci, City Campus, GPO Box 2476, Melbourne, Vic 3001, Australia
J Chromatogr A 2010 **1217** (1) 127
Comprehensive two-dimensional gas chromatography improves separation and identification of anabolic agents in doping control
The analysis of six anabolic agents (AAs) in doping control was examined by the application of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-TOFMS). A non-polar-polar column configuration with 0.2μm film thickness (d_f) second dimension (^{2}D) column was used an offered a much better spread of the components on ^{2}D when compared with the alternative 0.1μm d_f ^{2}D column. The proposed technique was tested on the "key" AA that the World Anti-Doping Agency (WADA) had listed at the low ng/ml levels (clenbuterol, 19-norandrosterone, epimethendiol, 17α-methyl-5α-androstane-3α,17β-diol, 17α-methyl-5β-androstane-3α,17β-diol and 3'-OH-stanozolol). Compounds spiked into urine blanks were obtained by solid-phase extraction, hydrolysis and liquid-liquid extraction. Prior to analysis they were converted to the corresponding trimethylsilyl (TMS) derivatives. The limit of detection (LOD) was below or equal to the minimum required performance limit (MRPL) of 2ng/ml defined by WADA, and the correlation coefficient was in the range from 0.995 to 0.999. The facilitates choice of an ion from the full mass spectra which demonstrates the least interference from the matrix and/or the best sensitivity. This is only possible if

full scan mass spectral data are available. The advantage of GCxGC over classical one-dimensional GC (1D GC), in respect of separation efficiency and sensitivity, was illustrated on a positive urine control sample at a concentration of 5ng/ml. The produced similarity with the in-house created TOFMS spectra library at this concentration was in the range from 822 to 932 (on the scale from 0 to 999). Full mass spectral information are recorded. Therefore, the procedure facilitates the retro-search of non-target compounds or new designer steroids. These cannot be detected with established GC-MS methods that use selected ion monitoring (SIM) mode

4 Peptides

Staub A, Giraud S, Saugy M, Rudaz S, Veuthey JL, Schappler J*// *Univ Geneva, Univ Lausanne, Sch Pharmaceut Sci, Blvd Yvoy 20, CH-1211 Geneva 4, Switzerland

Electrophoresis 2010 **31** (2) 388

CE-ESI-TOF/MS for human growth hormone analysis

Intact biomolecules such as proteins may be separated by the powerful analytical technique of capillary electrophoresis (CE). Furthermore, the coupling of CE with TOF/MS results in a very promising technique that may be employed to detect and identify proteins in different matrices. An efficient, rapid, and simple CE-ESI-TOF/MS technique is described for the analysis of endogenous human growth hormone and recombinant human growth hormone without sample preparation. Operational factors were optimized using an experimental design. The procedure was successfully utilised to distinguish human growth hormone from recombinant human growth hormone in unknown samples

6 CNS Agents

Lu JH, Wang S, Dong Y, Wang XB, Yang SM, Zhang JL, Deng J, Qin Y, Xu YX, Wu M, Ouyang GF*// *Sun Yat Sen Univ, Sch Chem & Chem Engn, Res Ctr Microextraction & Separation Technol, CN-510275 Guangzhou, Guangdong, Peoples Rep China

Anal Chim Acta 2010 **657** (1) 45

Simultaneous analysis of fourteen tertiary amine stimulants in human urine for doping control purposes by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) in combination with liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed and validated for the simultaneous screening and confirmation of the presence of fourteen tertiary amine stimulants in human urine. Pre-treatment of the urine samples was achieved with solid phase extraction (SPE) and liquid-liquid extraction (LLE) approaches. Under positive ion electrospray ionization (ESI) conditions the capillary temperature played a significant role in the signal abundances of the protonated molecules of cropropamide and crotethamide. Futhermore, comparison studies of two different pre-treatment approaches as well as the two ionization modes were performed. The LODs of the developed procedure for all the analytes were lower than the minimum required performance limit (MRPL) as set forth in the World Anti-Doping Agency (WADA) technical document for laboratories. Following oral administration of prolintane.HCl, human urine was successfully analyzed by the developed method. This demonstrates the applicability and reliability of the method for routine doping control analysis

Mazzarino M, De la Torre X, Mazzei F, Botre F*// *Antidoping Lab Rome, Largo Giulio Onesti 1, IT-00197 Rome, Italy

J Sep Sci 2009 **32** (20) 3562

Rapid screening of -adrenergic agents and related compounds in human urine for anti-doping purpose using capillary electrophoresis with dynamic coating

This paper presents a capillary electrophoresis method, developed for the detection, in human urine, of β-adrenergic agents and phenolalkylamines. The electrophoretic separation is achieved in less than 10 min and is based on the use of CEofix kit, for the dynamic capillary coating. The effects of accelerator buffer pH and separation voltage were investigated. The optimum buffer pH was found to be 2.5 for β2-agonists and 6.2 for β-blockers and phenoalkylamines with a separation voltage of 15 kV. Urine samples spiked with the compounds here studied were treated according to the standard procedure (SPE and evaporation to dryness) and analyzed by CE interfaced with an UV diode-array, set at 195 and 210 nm. The quantitative validation results, obtained analyzing samples at three different concentrations, show a good precision of peak areas that do not exceed 5% for intra-day assays and 10% for inter-day assays. Good linearity ($r^2 > 0.995$) was obtained within the 50-500 ng/ml concentration range. The qualitative validation data show a relative migration times (MTs) variation lower than 1%. The analytes were clearly distinguishable in urine, with LOD and LOQ in the range of 10-80 and 40-100 ng/ml, respectively

Pujos E, Cren-Olive C*, Paisse O, Flament-Waton MM, Grenier-Loustalot MF// *CNRS Service Central Anal - USR59, Chemin du Canal, FR-69360 Solaize, France

J Chromatogr B 2009 **877** (31) 4007

Comparison of the analysis of -blockers by different techniques

The analysis of 16 β-blockers (acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metipranolol, metoprolol, nadolol, oxprenolol, pindolol, propranolol, sotalol, timolol, and bupranolol) was performed using a comparison of three analytical techniques (ELISA, GC-MS, and LC-MS). Several sample-preparation methods were optimized for each procedure and afforded compounds of interest to be extracted from small urine samples (1-2.5 ml). The data allowed an assessment of the possibilities and the sensitivity of each method for use in doping tests. ELISA is useful as a rapid screening method but its selectivity is very poor and sensitivity the lowest. GC/MS and LC/MS facilitates confirmation procedures for the identification and quantification of the β-blockers with good sensitivity, accuracy and precision. LC-MS enabled the determination of the target analytes in the lower ng/ml range (0.53-2.23 ng/ml). The procedure was applied in the analysis of β-blockers in different urine samples

8 Recreational Drugs - General

Eichhorst JC, Etter ML, Rousseaux N, Lehotay DC*// *Saskatchewan Dis Control Lab, 3211 Albert St, Regina, Saskatchewan, Canada S4S 5W6

Clin Biochem 2009 **42** (15) 1531

Drugs of abuse testing by tandem mass spectrometry: A rapid, simple method to replace immunoassays

This research was conducted with the intention of replacing immunoassay screening for drugs of abuse (DOA) with a cost-effective tandem mass spectrometry method. In addition, it was performed tosubstantially expand the drugs of abuse assay menu. The requirement was to conduct high throughput DOA screening for 200 urine specimens/day for 40 drugs/metabolites. The total analysis time was required to be <5 min. UPLC chromatography, small particle size LC columns and fast scanning tandem mass spectrometrywas employed. Urine samples were enzymatically hydrolyzed, diluted and injected with isotopically labeled internal standards. Data produced were transferred by exporting reports as text files to a LIMS system followed by autocertification of the results. Forty different drugs were analysed by UPLC (ultra pressure liquid chromatography) with a run time of 5.2 min. Detection limits were below the cut-off values. Individual drug species instead of drug classes were identified; correlation with GC/MS was excellent. Consequently, a high throughput, robust procedure with good accuracy, precision and specificity was developed. The technique may also be employed for quantitative studies with simple modifications. An improved, high throughput, cost-effective protocol for drugs of abuse screening has been implemented. GC/MS confirmations were reduced or eliminated. The new technique is a viable alternative to the previous immunoassay procedure. Acceptable turn around times, an expanded drug menu, simplified sample preparation and analytical reliability demonstrates that this procedure is a desirable option in the clinical laboratory setting

Fritch D, Blum K, Nonnemacher S, Haggerty BJ, Sullivan MP, Cone EJ// 150 Webster St, Bethlehem, Pa 18015, USA

J Anal Toxicol 2009 **33** (9) 569

Identification and quantitation of amphetamines, cocaine, opiates, and phencyclidine in oral fluid by liquid chromatography-tandem mass spectrometry

Techniques for analysing multiple licit and illicit drugs and metabolites in oral fluid necessitate high sensitivity, specificity, and accuracy. Limited volumes are often available. Therefore, comprehensive methodology is required for simultaneous determination of multiple analytes in a single aliquot. Herein is described the validation of a semi-automated procedure for the simultaneous extraction, identification, and quantitation of 21 analytes in a single oral fluid aliquot. Target compounds include amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxy-amphetamine, 3,4-methylenedioxyethylamphetamine, pseudoephedrine, cocaine, benzoylecgonine, codeine, norcodeine, 6-acetylcodeine, morphine, 6-acetylmorphine, hydrocodone, norhydrocodone, dihydrocodeine, hydromorphone, oxycodone,

noroxycodone, oxymorphone, and phencyclidine. Oral fluid specimens were obtained with the Intercept device and extracted by solid-phase extraction (SPE). Drug recovery from the Intercept device averaged 84.3%, and SPE extraction efficiency averaged 91.2% for the 21 analytes. Analysis was accomplshed with liquid chromatography-tandem mass spectrometry in the positive electrospray mode using ratios of qualifying product ions within +/-25% of calibration standards. Matrix ion suppression ranged from -57 to 8%. Limit of quantitation ranged from 0.4 to 5 ng/ml using 0.2 ml of diluted oral fluid sample. Application of the protocol was illustrated by analysis of oral fluid specimens from drug abuse treatment patients. Oral fluid from 39 patients tested positive for various combinations of licit and illicit drugs and metabolites. Therefore, this validated technique is suitable for simultaneous measurement of 21 licit and illicit drugs and metabolites in oral fluid

Officer J// Scottish Police Serv Authority, Forensic Serv Edinburgh, 11 Howdenhall Rd, Edinburgh EH16 6TL, Scotland

Sci Justice 2009 **49** (4) 237

Trends in drug use of Scottish drivers arrested under Section 4 of the Road Traffic Act—a 10 year review

Research into Section 4 RTOA cases submitted to the Scottish Police Service Authority (SPSA) Forensic Science Laboratory in Edinburgh over a 12 year period has been performed. The main consideration of the analysis was to identify the most frequently encountered drugs and to deduce if there were any major drugs trends from the data collected. Data were divided into 3 groups as 1996-2000 (102 cases), 2003 (26 cases) and 2008 (295 cases) for analysis. The large increase in submissions was primarily as a result of the introduction of SPSA whereby the laboratory began to perform the analysis for all criminal and RTOA cases in Scotland. Preliminary results for the 8 drug groups (amphetamine and related compounds, benzodiazepines, cannabinoids, cocaine, methadone, methylamphetamine and related compounds, morphine and opiates) revealed a number of major trends. Cannabinoids were consistently present in 40-50% of cases, benzodiazepines more than doubled in frequency to over 80%, there was a significant increase in cases positive for morphine and methadone (up from less than 2% each to 31% and 23% respectively), there was an increase in the number of cases screening positive for opiates (19% to 29%), and the frequency of positive cases for cocaine, amphetamine and methylamphetamine remained unaltered (approximately 22%, 6% and 5% respectively). A significant finding was the very large increase in polydrug use. The number of cases positive for 4 or more drug groups increased from 4% in 1996-2000 to 25% in 2008. By comparison, in the 1996-2000 group 72% of cases were only positive for one drug group compared to 17% in 2008. In those instances which were negative for all 8 drug groups, screening for potentially impairing prescription and over the counter medicines was performed. Most frequently discovered medicines were sedatives, sedative antidepressants, sedative antihistamines and antiemetics. These were often noted to be in conjunction with alcohol which was below the legal limit for driving

9 Stimulants

Bucelli F, Fratini A, Bavazzano P, Comodo N// Univ Florence, Dipt Sanita Pubblica, Viale Morgagni 48, IT-50134 Florence, Italy

J Chromatogr B 2009 **877** (31) 3931

Quantification of drugs of abuse and some stimulants in hair samples by liquid chromatography-electrospray ionization ion trap mass spectrometry

The analysis of drugs of abuse (cocaine and benzoylecgonine, opiates) and some stimulants in human hair was analysed both qualitatively and quantitatively by a procedure which was developed and validated. Hair samples were incubated with phosphate buffer (pH 5.0) as the extraction medium, extracted with Bond Elut Certify cartridges and analyzed by LC-MS-MS and LC-MS^3 as confirmation for positive results. The technique was shown to be specific, accurate and precise across the calibration range (0.1-30 ng/mg) where good linearity was demonstrated. Total extraction recovery, intra-assay accuracy and precision, limits of detection and limits of quantitation were evaluated. The procedure was successfully employed in the analysis of hair samples collected from drug abusers. It proved applicable for routine analytical applications in the Antidoping Laboratory of Public Health Laboratory

Fernandez MDR, Wille SMR, Samyn N, Wood M, Lopez-Rivadulla M, De Boeck G// Federal Publ Serv Justice, Natl Inst Criminalistics & Criminology, Chaussee Vilvorde 100, BE-1120 Brussels, Belgium

J Anal Toxicol 2009 **33** (9) 578

High-throughput analysis of amphetamines in blood and urine with online solid-phase extraction-liquid chromatography-tandem mass spectrometry

An automated online solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS-MS) procedure for the determination of amphetamines in blood and urine was developed and validated. Chromatographic separation was accomplished with a NUCLEODUR Sphinx RP column employing an LC gradient (a mixture of 10 mM ammonium formate buffer and acetonitrile), resulting in the elution of amphetamine, methamphetamine, MDMA, MDA, MDEA, PMA, and ephedrine within 11 min. The procedure was fully validated in accordance with international guidelines and employed only 100 of blood and 50 µl of urine. The technique demonstrated excellent intra- and interassay precision (relative standard deviation < 11.2% and bias < 13%) for two external quality control samples (QC) for both matrices and three and two 'in house' QCs for blood and urine, respectively. Responses were linear over the investigated range (r(2) > 0.99, 2.5-400 µg/l for blood and 25-1000 µg/l for urine). Limits of quantification were determined to be 2.5 µg/l for blood and 25 µg/l for urine. Limits of detection ranged from 0.05 to 0.5 µg/l for blood and 0.25 to 2.5 µg/l for urine, depending on the compound. Analytes and processed samples were shown to be stable (in the autosampler for at least 72 h and after three freeze/thaw cycles) and no notable matrix effects were observed for all analytes. In addition, no carryover was demonstrated after the analysis of high concentration samples (15,000 µg/l). Subsequently, the protocol was employed with authentic blood and urine samples from forensic cases which covered a broad range of concentrations. The validation data and actual sample analyses illustrated that this technique is rugged, precise, accurate, and well-suited for routine analysis; more than 72 samples were analyzed non-stop in 24 h with minimum sample handling. The association of high-throughput online SPE and the well-known sensitivity and selectivity assured by MS-MS negated the impediment associated with the sample preparation requirements. This resulted in increased sensitivity, accuracy, and precision

John H, Binder T, Hochstetter H, Thiermann H// Bundeswehr Inst Pharmacol & Toxicol, Neuherbergstr 11, DE-80937 Munich, Germany

Anal Bioanal Chem 2010 **396** (2) 751

LC-ESI MS/MS quantification of atropine and six other antimuscarinic tropane alkaloids in plasma

The simultaneous determination of seven natural and semisynthetic tropane alkaloids in plasma [atropine (*d*-hyoscyamine/l-hyoscyamine), cocaine, homatropine, ipratropium, littorine, *N*-butylscopolamine, and scopolamine] has been achieved by the development and validation of a quantitative liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI MS/MS) technique. Plasma and serum samples were precipitated for deproteinization (recovery 88-94%). Subsequently, reversed-phase-based liquid chromatography was employed prior to positive electrospray ionization for detection by multiple reaction monitoring using a linear ion trap quadrupole mass spectrometer. Quantification of compounds was achieved using cocaine-d_3 as an internal standard suitable and reliable for robust, precise (coefficient of variation 2-13%), and accurate (87-122%) measurement within a linear range of 3 orders of magnitude (0.05-50 ng/ml plasma). The technique was initailly employed for stability studies in phosphate-buffered saline, human serum, and rabbit serum. Each alkaloid was incubated separately and samples were obtained at set time periods. Supernatants of diverse alkaloids at corresponding time points were pooled and subjected to simultaneous LC-ESI MS/MS quantification. This combinatorial procedure facilitated analysis of the stability of samples with a drastically reduced number of chromatographic runs. In rabbit serum, all tropane alkaloids examined were metabolized significantly within minutes to hours, with the exception of the stable semisynthetic compounds ipratropium and *N*-butylscopolamine. By contrast, in equal concentrations of human serum, no degradation was noted for any of the compounds, with the exception of cocaine. Enzymes involved in the metabolism are discussed

Viel G, Nalesso A, Cecchetto G, Montisci M, Ferrara SD*// *Dept Environm Med & Publ Hlth, Section Legal Med, Forensic Toxicol & Antidoping Unit, Via Falloppio 50, IT-35121 Padua, Italy

Forensic Sci Int 2009 **193** (1-3) 79

Stability of cocaine in formalin solution and fixed tissues

Embalming of bodies and formalin fixation of tissues are common. However, they may pose a problem for the forensic scientist if a drug has been the cause of death and where the only available specimens to be analyzed have been treated by formalin-fixation. It has previously been shown that during fixation, xenobiotics are extracted into formalin depending on tissue and fixing solution characteristics. In addition, in some instances, formalin may react with the analyte resulting in the production of new chemical entities. In respect of cocaine and its metabolites, Cingolani *et al*. have demonstrated that formalin-fixation extracts benzoylecgonine (BE) from tissues and that BE is stable in the fixing solution. However, the stability and kinetic properties of cocaine remain so far unexplored. Data presented here demonstrate that in buffered formalin

(pH 7.4), cocaine is hydrolyzed to BE in agreement with a pseudo first-order reaction kinetic (half-life time approximately 7 days). However in unbuffered formalin (pH approximately 3.5) it is relatively stable over a period of 30 days. An investigation of brain and liver samples following different fixation times suggests that during fixation an extraction process occurs for both compounds. However, it would appear that the extraction is more efficient in the liver than in the brain, probably as a result of the greater lipophilicity of the brain tissue. The data indicate that formalin-fixed tissues and their fixing solutions may be used for cocaine analysis but only if a short time period has elapsed since commencement of fixation. The rapid extraction of cocaine into formalin and its concomitant hydrolysis to BE in buffered formalin may preclude the identification of cocaine in both tissues and formalin solution at 15-30 days following fixation. In addition, the unpredictable extraction rate of both analytes, along with the hydrolysis of cocaine into BE significantly affects tissue concentrations. Therefore, the interpretation of quantitative results is complicated

10 Hallucinogens

Belal T, Awad T, Clark CR*, DeRuiter J// *Auburn Univ, Harrison Sch Pharm, Dept Pharmacal Sci, Auburn, Al 36849, USA

J Chromatogr Sci 2009 **47** (5) 359

GC-MS evaluation of a series of acylated derivatives of 3,4-methylenedioxymethamphetamine

Gas chromatography-mass spectrometry (GC-MS) was performed to analyse a series of acylated derivatives of 3,4-methylenedioxy-methamphetamine (3,4-MDMA). Perfluoroalkyl amides of 3,4-MDMA demonstrate the lowest GC retention, while the aromatic amides such as the benzamide exhibit the greatest retention on the dimethylpolysiloxane stationary phase (Rtx-1). Mass spectral properties of the acetyl, propionyl, and butyryl derivatives all demonstrate a base peak at *m/z* 58 which is the base peak for the underivatized 3,4-MDMA. All acylated derivatives produce mass spectral information (*m/z* 162) to identify the three-carbon side chain for 3,4-MDMA. Perfluoroalkyl amides produce several unique mass spectral fragments for specific identification of 3,4-MDMA. MS fragmentation pathways are illustrated and validated using analogous deuterated derivatives. The combination of excellent chromatographic properties and unique mass spectral fragments facilitates the perfluoroalkyl amides to provide maximum specific structural information in the GC-MS analysis of 3,4-MDMA

Bjornstad K, Helander A, Hulten P, Beck O*// *Karolinska Univ Hosp Solna, L7:05, SE-17176 Stockholm, Sweden

J Anal Toxicol 2009 **33** (9) 604

Bioanalytical investigation of asarone in connection with acorus calamus oil intoxications

Preparations of the plant *Acorus calamus* (*A. calamus*, calamus or sweet flag) are available over the internet and sold as being hallucinogenic. In 2003-2006, the Swedish Poisons Information Centre received inquiries about 30 clinical cases of intentional intoxication with *A. calamus* products. A study has been made to identify α- and β-asarone, considered active components of *A. calamus*, and their metabolites in urine samples collected in seven of these cases. To assist in the identification of asarone biotransformation products, a calamus oil preparation was incubated with the fungus *Cunninghamella elegans*, which has been employed as a microbial model of mammalian drug metabolism. Gas chromatography-mass spectrometry (GC-MS) analysis in selected ion monitoring mode demonstrated α-asarone in five urine samples at concentrations ranging between approximately 11 and 1150 μg/l and β-asarone in four of those at approximately 22-220 μg/l. A previously identified asarone metabolite, *trans*-2,4,5-trimethoxycinnamic acid (*trans*-TMC), was confirmed in the fungal broth with liquid chromatography-tandem mass spectrometry. *Cis*-TMC was tentatively identified in the human urine samples. A hydroxylated asarone metabolite was identified both in fungal broth and urine samples with GC-MS. Interestingly, no evidence for the presence of 2,4,5-trimethoxyamphetamine, claimed as a hallucinogenic component of *A. calamus*, was demonstrated. The principal clinical symptom reported by the patients was prolonged vomiting that sometimes lasted more than 15 h

Choi H, Baeck S, Kim E, Lee S, Jang M, Lee J, Choi H, Chung H// Natl Inst Sci Invest, Dept Forensic Sci, Narcotic Anal Div, 331-1 Shinwol 7 dong, Yangcheon gu, Seoul 158 707, South Korea

Sci Justice 2009 **49** (4) 242

Analysis of cannabis in oral fluid specimens by GC-MS with automatic SPE

In Korea, methamphetamine (MA) is the most commonly abused drug followed by cannabis. Usually, MA analysis is performed on both urine and hair samples whereas cannabis analysis is of urine samples only. Oral fluid has become increasingly popular as an alternative specimen in the field of driving under the influence of drugs (DUID) and workplace drug testing. However, its application has not been expanded to drug analysis in Korea. Oral fluid is easy to collect and handle and is capable of providing an indication of recent drug abuse.Presented, is an analytical procedure utilising GC-MS to determine tetrahydrocannabinol (THC) and its main metabolite 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in oral fluid. The validated technique was employed with oral fluid specimens obtained from drug abuse suspects and the results were compared with those from urine. The stability of THC and THC-COOH in oral fluid stored in different containers was also examined. Oral fluid specimens from 12 drug abuse suspects, submitted by the police, were obtained by direct expectoration. Samples were screened with microplate ELISA. Confirmation was accomplished from samples extracted using automated SPE with mixed-mode cation exchange cartridge, derivatized and analyzed by GC-MS using selective ion monitoring (SIM). Concentrations of THC and THC-COOH in oral fluid showed large variation and the results from oral fluid and urine samples from cannabis abusers did not exhibit any correlation. Detailed information about time interval between drug use and sample production is required to interpret the oral fluid results properly. Furthermore, an investigation in respect of the detection time window of THC and THC-COOH in oral fluid is necessary to replaceoral fluid with urine in drug testing

David GE, Hibbert DB, Frew RD, Hayman AR*// *Univ Otago, Dept Chem, POB 56, Dunedin 9054, New Zealand

Aust J Chem 2010 **63** (1) 22

Significant determinants of isotope composition during HI/P-red synthesis of methamphetamine

Methamphetamine HCl was produced with three variations of the hydriodic acid/red phosphorus (HI/P-red) synthetic route. A Plackett-Burman experimental design was utilised to analyse how reaction parameters affect the isotopic composition of the product. Isotope ratio mass spectrometry data indicated that only the source of precursor ^{13}C was significant in determining product $\delta^{13}C$ (the manufacturer, reaction temperature, time, scale, and source of HI were not significant). The precursor was also the main determining factor of product δ^2H, with smaller contributions from the HI source for one method. From precursor to product, substantial δ^2H depletion occurred for most samples. Deuterium nuclear magnetic resonance spectroscopy (^2H NMR) was employed to investigate the specific site of this effect. A significant fraction of deuterium was noted only at the benzylic position, the site of hydrogen addition during synthesis. Methamphetamine produced from ephedrine was demonstrated to be depleted in this position

De Backer B, Debrus B, Lebrun P, Theunis L, Dubois N, Decock L, Verstraete A, Hubert P, Charlier C// Univ Liege, CHU Sart Tilman, Serv Toxicol Clin Medicol Environnement & Entreprise, BE-4000 Liege, Belgium

J Chromatogr B 2009 **877** (32) 4115

Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material

GC is routinely employed in the analysis of cannabis samples, e.g. in forensic chemistry. However, as this procedure relies on heating of the sample, acidic forms of cannabinoids are decarboxylated into their neutral counterparts. By contrast, HPLC facilitates the determination of the original composition of plant cannabinoids by direct analysis. In the literature, several HPLC procedures are described but most of them fail to separate efficiently all the cannabinoids or have not been validated according to general guidelines. By employing an innovative methodology for modelling chromatographic responses, a simple and accurate HPLC/DAD procedure has been developed for the quantification of major neutral and acidic cannabinoids present in cannabis plant material [Δ^9-tetrahydrocannabinol (THC), THC acid (THCA), cannabidiol (CBD), CBD acid (CBDA), cannabigerol (CBG), CBG acid (CBGA) and cannabinol (CBN)]. Δ^8-Tetrahydrocannabinol (Δ^8-THC) was determined qualitatively. In order to find optimal chromatographic analytical conditions, predictive multilinear models were developed and utilised. The procedure was validated following an approach employing accuracy profiles based on β-expectation tolerance intervals for the total error measurement, and assessing the measurements uncertainty. This analytical technique may be employed for diverse applications, e.g. plant phenotype determination, evaluation of psychoactive potency and control of material quality

Inagaki S, Makino H, Fukushima T, Min JZ, Toyo'oka T*// *Univ Shizuoka, Sch Pharmaceut Sci, Lab Anal & Bioanal Chem, 52-1 Yada, Suruga ku, Shizuoka 422 8526, Japan

Biomed Chromatogr 2009 **23** (12) 1245

Rapid detection of ketamine and norketamine in rat hair using micro-pulverized extraction and ultra-performance liquid chromatography-electropsray ionization mass spectrometry

A new method for the rapid and simultaneous detection of ketamine and its major metabolite, norketamine, in rat hair has been developed by combining micropulverized extraction and ultraperformance liquid chromatography-electrospray ionization mass spectrometry. By using reversed-phase UPLC, ketamine and norketamine were well separated within 2 min. Using ketamine-dosed rat hair, the conditions for micropulverized extraction were optimized, and the limits of detection and quantification of the developed method were found to be 1.7 and 5.7 pg/mg hair for ketamine, respectively. The precisions achieved with this method were slightly better than that obtained with conventional acidic methanol extraction method. Using this proposed method, analysis of the washed rat hair could be completed within 16-17 min. This method is expected to be applied for the analysis of the hair samples of not only rats but also ketamine abusers

Kugelberg FC, Holmgren A, Eklund A, Jones AW*// *Natl Board Forensic Med, Dept Forensic Genet & Forensic Toxicol, Artillerigatan 12, SE-58758 Linkoping, Sweden

Int J Legal Med 2010 **124** (1) 1

Forensic toxicology findings in deaths involving -hydroxybutyrate

In a series of GHB-related deaths (n = 49), the cncentrations of the illicit drug γ-hydroxybutyrate (GHB) were analysed in femoral venous blood and urine obtained at autopsy. Analysis of GHB was accomplished with gas chromatography after conversion to γ-butyrolactone and quantitation of the latter with a flame ionization detector. The cutoff concentration of GHB in femoral blood or urine for reporting positive results was 30 mg/l. The deceased were mainly young men (86%) aged 26.5 +/- 7.2 years (mean +/- SD), and the women (14%) were about 5 years younger at 21.4 +/- 5.0 years. The mean, median, and highest concentrations of GHB in femoral blood (n = 37) were 294, 190, and 2,200 mg/l, respectively. The mean urine-to-blood ratio of GHB was 8.8, and the median was 5.2 (n = 28). In 12 cases, the concentrations of GHB in blood were negative (<30 mg/l) when the urine contained 350 mg/l on average (range 31-1,100 mg/l). Considerable poly-drug use was present in these GHB-related deaths. Substances included ethanol (18 cases), amphetamine (12 cases), and various prescription medications (benzodizepines, opiates, and antidepressants) in other cases. Interpretation of the levels of GHB in postmortem blood is complicated due to concomitant use of other psychoactive substances, variable degree of tolerance to centrally acting drugs, and the absence of reliable information about survival time after use of the drug

McIlhenny EH, Pipkin KE, Standish LJ, Wechkin HA, Strassman R, Barker SA*// *Louisiana State Univ, Sch Veterinary Med, Dept Comparative Biomed Sci, Skip Bertman Dr & River Rd, Baton Rouge, La 70803, USA

J Chromatogr A 2009 **1216** (51) 8960

Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca by liquid chromatography-electrospray ionization-tandem mass spectrometry

The simultaneous quantitation of 11 compounds potentially found in the increasingly popular Amazonian botanical medicine and religious sacrament ayahuasca has been achieved using a direct injection/liquid chromatography-electrospray ionization-tandem mass spectrometry protocol. The technique employs a deuterated internal standard for quantitation and facilitates rapid detection of the alkaloids by a simple dilution assay without extraction procedures. In addition, the procedure indicates a high degree of specificity for the analytes in question, as well as low limits of detection and quantitation despite using samples for analysis that had been diluted up to 200:1. The technique also appears to eliminate potential matrix effects. Technique bias for each analyte, examined over a range of concentrations, was also determined as was inter- and intra-assay variation. Analysis of three different ayahuasca preparations was performed. The procedure might facilitate the study of ayahuasca in clinical and ethnobotanical research in addition to forensic examinations of ayahuasca preparations

Schwilke EW, Schwope DM, Karschner EL, Lowe RH, Darwin WD, Kelly DL, Goodwin RS, Gorelick DA, Huestis MA*// *NIH/NIDA, Intramural Res Program, Biomed Res Ctr, 251 Bayview Blvd, Suite 200, Rm 05A-721, Baltimore, Md 21224, USA

Clin Chem 2009 **55** (12) 2180

^{9}-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC plasma pharmacokinetics during and after continuous high-dose oral THC

Δ^{9}-tetrahydrocannabinol (THC) is the predominant psychoactive compound in cannabis. It is also an active component of cannabinoid pharmacotherapy. No plasma pharmacokinetic data following repeated oral THC administration are available. Six adult male daily cannabis smokers resided on a closed clinical research unit. Oral THC capsules (20 mg) were administered every 4-8 h in escalating total daily doses (40-120 mg) for 7 days. Free and glucuronidated plasma THC, 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) were quantified by 2-dimensional GC-MS during and after dosing. Free plasma THC, 11-OH-THC, and THCCOOH concentrations 19.5 h after admission (before controlled oral THC dosing) were mean 4.3 (SE 1.1), 1.3 (0.5), and 34.0 (8.4) µg/l, respectively. Free 11-OH-THC and THCCOOH increased steadily during oral dosing. However, THC did not. Mean peak plasma free THC, 11-OH-THC, and THCCOOH concentrations were 3.8 (0.5), 3.0 (0.7), and 196.9 (39.9) µg/l, respectively, 22.5 h after the last dose. *Escherichia coli* β-glucuronidase hydrolysis of 264 cannabinoid specimens demonstrated statistically significant increases in THC, 11-OH-THC, and THCCOOH concentrations ($P > 0.001$). However, conjugated concentrations were underestimated because of incomplete enzymatic hydrolysis. Plasma THC concentrations persisted at <1 µg/l for at least 1 day after daily cannabis smoking and also after cessation of multiple oral THC doses. This is the first report detailing free plasma THC concentrations following multiple high-dose oral THC throughout the day and night, and after *Escherichia coli* β-glucuronidase hydrolysis. These data should assist in the interpretation of plasma THC concentrations after multiple oral doses

Strano-Rossi S, Botre F, Bermejo AM, Tabernero MJ// Federazione Medico Sportiva Italiana, Antidoping Lab Rome, Largo Giulio Onesti 1, IT-00197 Rome, Italy

Forensic Sci Int 2009 **193** (1-3) 95

A rapid method for the extraction, enantiomeric separation and quantification of amphetamines in hair

The determination and chiral separation of amphetamines and related designer drugs in hair samples has been achieved with a rapid and sensitive procedure. Compounds were extracted from hair matrix by a 30 min treatment with a saturated carbonate buffer at pH 10 under ultrasonication. Trifluoroacetyl-prolyl chloride, commercial chiral derivatizing agent, is then added to the solution. Subsequently,the solution is directly extracted with hexane and analyzed by GC/MS in SIM mode. *R* and *S* isomers of amphetamine, methamphetamine, MDA, MDMA and MDEA may be separated and detected with a limit of detection of 0.1 ng/mg for amphetamine, methamphetamine and MDA, and of 0.2 ng/mg for MDMA and MDEA. The procedure was employed with 12 samples from suspected amphetamines abusers. It indicated the presence of both isomers of amphetamine and MDMA in one sample (27 and 1.5 ng/mg, respectively) and of MDMA in further eight samples, in concentrations ranging from traces to 2.7 ng/mg. No differences were noted in the disposition of different isomers in hair

Tsujikawa K, Kuwayama K, Miyaguchi H, Kanamori T, Iwata YT, Inoue H// Natl Res Inst Police Sci, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277 0882, Japan

Forensic Sci Int 2009 **193** (1-3) 106

Degradation of *N*-hydroxy-3,4-methylenedioxymethamphetamine in aqueous solution and its prevention

A a lesser known psychedelic drug that has recently appeared in the Japanese illicit drug market is *N*-hydroxy-3,4-methylenedioxymethamphetamine (*N*-OH-MDMA). *N*-hydroxy-3,4-methylenedioxyamphetamine (*N*-OH-MDA) has a similar structure and exhibits instability in neutral-to-basic aqueous solution. Therefore, it was predicted that *N*-OH-MDMA would also degrade in aqueous solution. A number of aims were proposed. (i) Investigation of the degradation of *N*-OH-MDMA in aqueous solution and its prevention. (ii) Identification of the degradation products. (iii) Determination of the pKa for the conjugate acid of *N*-OH-MDMA. (iv) Evaluation of liquid-liquid extraction recovery. *N*-OH-MDA was also included in some of these studies. *N*-OH-MDMA degraded to 14.9% of initial concentration after 2 h storage in pH 10 buffer solution at 22 °C. Degradation was completely prevented for at least for 2 h by the addition of L-ascorbic acid, a strong reactive oxygen scavenger. The results suggest that reactive oxygen species in alkaline solution were involved in *N*-OH-MDMA degradation. *N*-OH-MDA, α-methyl-(*N*-methylene)-3',4'-methylenedioxybenzeneethanamine and 3',4'-methylenedioxyphenyl-2-propanone oxime were noted as degradation products of *N*-OH-MDMA in alkaline solution. The pKa for the conjugate acid of *N*-OH-MDMA was determined by

titration to be 5.52. This was much lower than that reported for 3,4-methylenedioxymethamphetamine (pKa = 10.38). Excellent recoveries for *N*-OH-MDMA and *N*-OH-MDA (>98%) were accomplished by extraction with ethyl acetate or chloroform from a basic buffer (pH 10) solution containing 0.1% L-ascorbic acid

Westin AA, Huestis MA, Aarstad K, Spigset O// St Olavs Univ Hosp, Dept Clin Pharmacol, Olav Kyrres gate 17, NO-7006 Trondheim, Norway
J Anal Toxicol 2009 **33** (9) 610
Urinary excretion of 11-nor-9-carboxy- ^{9}-tetrahydrocannabinol in a pregnant woman following heavy, chronic cannabis use
Identifying fresh consumption of drugs-of-abuse from residual drug excretion may be difficult, particularly in cases of chronic drug usage and for drugs with long elimination half-lives such as cannabis. In the present case, cannabis was demonstrated in the urine of a young pregnant woman following heavy and chronic cannabis use. She received a warning that if she persisted using cannabis whilst pregnant, she would be required to be hospitalized. Serial urine testing was conducted at 2-7-day intervals. Urinalysis revealed that 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) concentrations, measured by liquid chromatography-mass spectrometry, declined from 348 to 3.9 ng/ml over a protracted period of 12 weeks (84 days). Several algorithms to identify new drug intake were applied during this time course. Most suggested that the woman persisted in smoking cannabis following the first urine test. The woman denied any abuse following the first specimen collection. Retrospective analysis of her THCCOOH excretion profile supported her story. Algorithms for identification of new drug intake have been validated for occasional cannabis users only. Caution is advisable when interpreting urine test results from heavy, chronic cannabis users, particularly when serious consequences are involved

11 Narcotics

Bo XJ, Xie WL, Ndamanisha JC, Bai J, Guo LP*// *NE Normal Univ, Fac Chem, CN-130024 Changchun, Peoples Rep China
Electroanalysis 2009 **21** (23) 2549
Electrochemical oxidation and detection of morphine at ordered mesoporous carbon modified glassy carbon electrodes
In this work, three ordered mesoporous carbons (OMCs) with different structural parameters were synthesized by a simple variation of the hydrothermal temperature of the silica templates (SBA-15). X-ray diffraction and nitrogen adsorption-desorption results show these OMCs exhibit an ordered 2D hexagonal mesostructure with tunable pore diameter. OMC-modified glassy carbon electrodes exhibit efficient electrocatalytic reactivity toward oxidation of morphine (MO). The amperometric detection of MO in pH 7.0 phosphate buffered saline at +0.39 V *versus* Ag/AgCl is the lowest potential reported to-date. A linear range from 0.2 to 197.6 μM and a detection limit of 0.03 μM MO were obtained.

Bonanno LM, DeLouise LA*// *Univ Rochester Med Ctr, Dept Dermatol, 601 Elmwood Ave, Box 697, Rochester, NY 14642, USA
Anal Chem 2010 **82** (2) 714
Tunable detection sensitivity of opiates in urine *via* a label-free porous silicon competitive inhibition immunosensor
Presently, there is a requirement for laboratory-based high-throughput and reliable point-of-care drug screening techniques. In this paper, a chip-based label-free porous silicon (PSi) photonic sensor is described for detecting opiates in urine. This method facilitates a cost-effective alternative to conventional labeled drug screening immunoassays with potential for translation to multiplexed analysis. Marked influences of surface chemistry and competitive binding assay procedure on the sensitivity of opiate detection were investigated. The ability to tune sensitivity and detection range over approximately 3 orders of magnitude (18.0 nM to 10.8 μM) was accomplished by varying the applied urine specimen volume (100-5 μl) resulting in systematic shifts in the competitive binding response curve. A detection range (0.36-4.02 μM) of morphine in urine (15 μl) was designed to encompass the current positive cutoff value (1.05 μM morphine) in medical opiate urine screening. Desirable high cross-reactivity to oxycodone, in addition to other common opiates, morphine, morphine-3-glucuronide, 6-acetyl morphine, illustrates an advantage over current commercial screening assays while low interference with cocaine metabolite was maintained. The research uniquely demonstrates PSi sensor technology as an inexpensive, rapid, and reliable drug screening technology. In addition, the versatile surface chemistry developed may be employed on a range of solid-supported sensors to conduct competitive inhibition assays

Morello DR, Cooper SD, Panicker S, Casale JF// US Drug Enforcement Admin, Special Testing & Res Lab, 22624 Dulles Summit Court, Dulles, Va 20166, USA
J Forensic Sci 2010 **55** (1) 42
Signature profiling and classification of illicit heroin by GC-MS analysis of acidic and neutral manufacturing impurities
The illicit manufacture of heroin results in the formation of trace level acidic and neutral impurities. These impurities are detectable in illicit heroin and provide valuable information about the manufacturing process used. The isolation, derivatization, and semiquantitative analysis of neutral and acidic heroin manufacturing impurities by programmed temperature vaporizing injector-gas chromatography-mass spectrometry (PTV-GC-MS) is described. Trace acidic and neutral heroin impurities were isolated from basic fractions using liquid-liquid extraction. Extracted impurities were treated with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide followed by PTV-GC-MS analyses. Semiquantitative data were obtained using full scan mass spectrometry utilizing unique ions or ion combinations for 36 trace impurities found in crude and/or highly refined heroin samples. Minimum detection limits for acidic and neutral impurities were estimated to be at the 10^{-7} level relative to total morphine. Over 500 authentic heroin samples from South America, Mexico, Southwest Asia, and Southeast Asia were analyzed. Classification of illicit heroin based on the presence or absence and relative amounts of acidic and neutral impurities is presented

Papaseit E, Corrales E, Stramesi C, Vall O, Palomeque A, Garcia-Algar O*// *Hosp Mar, IMIM, URIE, Paediat Serv, Pg Maritim 25-29, ES-08003 Barcelona, Spain
Acta Paediatr 2010 **99** (2) 162
Postnatal methadone withdrawal syndrome: Hair analysis for detecting chronic exposure (Letter)
No abstract available

12 Forensics

Li JM, Jiang Y*// *Hebei Med Univ, Sch Pharm, Dept Pharmaceut Anal, 361 East Zhongshan Rd, Shijiazhuang, Peoples Rep China
Biomed Chromatogr 2010 **24** (2) 186
Rapid and sensitive determination of strychnine and brucine in human urine by capillary electrophoresis with field-amplified sample stacking
A simple, rapid, sensitive and low-cost method using capillary electrophoresis (CE) coupled with field-amplified sample stacking (FASS) has been developed and validated for the simultaneous determination of strychnine and brucine residues in human urine. Before sample loading, a water plug (3.5 kPa, 3 s) was injected to contain sample cations and to permit FASS. Electrokinetic injection at a voltage (20 kV, 25 s) was then used to introduce cations. Separation was performed using 20 mM acetate buffer (pH 3.8) with an applied voltage of 20 kV. The calibration curves were linear over a range of 8.00-2.56 ∞ 10^2 ng/ml (r = 0.9995) for strychnine and 10.0-3.20 x 10^2 ng/ml (r = 0.9999) for brucine. Extraction recoveries in urine were greater than 79.6 and 82.8% for strychnine and brucine, respectively, with an RSD of less than 4.9%. The detection limits (signal-to-noise ratio 3) for strychnine and brucine were 2.00 and 2.50 ng/ml, respectively. A urine sample from one healthy female volunteer (26 years old, 50 kg) was pretreated and analyzed. Strychnine and brucine levels in urine could be detected 24 h after administration. On these grounds, this method was feasible for application to preliminary screening of trace levels of abused drugs for both doping control and forensic analysis

Liotta E, Gottardo R, Bertaso A, Polettini A*// *Univ Verona, Dept Med & Publ Hlth, Unit Forensic Med, Policlin Borgoroma, Ple LA Scuro 10, IT-37134 Verona, Italy
J Mass Spectrom 2010 **45** (3) 261
Screening for pharmaco-toxicologically relevant compounds in biosamples using high-resolution mass spectrometry: A 'metabolomic' approach to the discrimination between isomers
High-resolution mass spectrometry (HRMS) enables the identification of a chemical formula of small molecules through the accurate measurement of mass and isotopic pattern. However, the identification of an unknown compound starting from the chemical formula requires additional tools: (1) a database associating chemical formulas to compound names and (2) a way to discriminate between isomers. The aim of this present study is to evaluate the ability of a novel 'metabolomic' approach to reduce the list of candidates with identical chemical formula. Urine/blood/hair samples collected from real positive cases were submitted to a screening procedure using ESI-MS-TOF

(positive-ion mode) combined with either capillary electrophoresis or reversed phase liquid chromatography (LC). Detected peaks were searched against a Pharmaco/Toxicologically Relevant Compounds database (ca 50,500 compounds and phase I and phase II metabolites) consisting of a subset of PubChem compounds and a list of candidates was retrieved. Then, starting from the mass of unknown, mass shifts corresponding to pre-defined biotransformations (e.g. demethylation, glucuronidation, etc.) were calculated and corresponding mass chromatograms were extracted from the total ion current (TIC) in order to search for metabolite peaks. For each candidate, the number of different functional groups in the molecule was automatically calculated using E-Dragon software (Talete srl, Milan, Italy). Then, the presence of metabolites in the TIC was matched with functional groups data in order to exclude candidates with structures not compatible with observed biotransformations (e.g. loss of methyl from a structure not bearing methyls). The procedure was tested on 108 pharmaco-toxicologically relevant compounds (PTRC) and their phase I metabolites were detected in real positive samples. The mean list length (MLL) of candidates retrieved from the database was 7.01 +/- 4.77 (median, 7; range, 1-28) before the application of the 'metabolomic' approach, and after the application it was reduced to 4.08 +/- 3.11 (median 3, range 1-17). HRMS allows a much broader screening for PTRC than other screening approaches (e.g. library search on mass spectra databases). The 'metabolomic' approach enables the reduction of the list of candidate isomers

McIntyre IM, Sherrard JL, Nelson CL// San Diego County Med Examiners Office, San Diego, Ca 92123, USA
J Anal Toxicol 2009 **33** (9) 615
Oxymorphone-involved fatalities: A report of two cases
Increased awareness suggests that illicit opiate abusers are using the narcotic oxymorphone (Opana) by inhalation. However, many current laboratory screening methods fail to detect oxymorphone in blood or urine. Therefore, biological specimens with low to moderate concentrations of oxymorphone may well go undetected. The circumstances, pathology findings, and toxicology results of two fatalities involving oxymorphone are presented. A gas chromatography-mass spectrometry (GC-MS) technique for opiate analysis is described in detail and was able to detected, confirm, and quantify oxymorphone in both subjects. The blood concentrations were 0.05 mg/l (50 µg/l) and 0.12 mg/l (120 µg/l)

Song JZ, Han QB, Qiao CF, But PPH, Xu HX*// *Hong Kong Jockey Club Inst Chinese Med, Bio-Informat Ctr, Unit 703, 7th Floor, 2 Science Park West Ave, Shatin, Hong Kong, Peoples Rep China
Phytochem Anal 2010 **21** (2) 137
Development and validation of a rapid capillary zone electrophoresis method for the determination of aconite alkaloids in aconite roots
Aconites, with aconite alkaloids as the major therapeutic and toxic components, are used for the treatment of analgesic, antirheumatic and neurological symptoms. Quantification of the aconite alkaloids is important for the quality control of aconite-containing drugs. The objective was to establish a validated capillary zone electrophoresis (CZE) method for the simultaneous determination of six major alkaloids, namely aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine and benzoylhypaconine, in crude and processed aconite roots. The CZE method was optimised and validated using a stability-indicating method. The optimised running buffer was a mixture of 200 mm Tris, 150 mm perchloric acid and 40% 1,4-dioxane (pH 7.8) with the capillary thermostated at 25 °C. Using the optimised method, six aconite alkaloids were well separated. The established method showed good precision, accuracy and recovery. Contents of these alkaloids in crude and processed aconites were determined and it was observed that the levels of individual alkaloids varied between samples. The developed CZE method was reliable for the quality control of aconites contained in herbal medicines. The method could also be used as an approach for toxicological studies

Stoel RD, Bolck A// Netherlands Forensic Inst, Dept Digital Technol & Biometry, The Hague, The Netherlands
J Forensic Sci 2010 **55** (1) 213
Correction to Tzidony and Ravreby (1992): A statistical approach to drug sampling: A case study
In 1992, Tzidony and Ravreby presented a confidence interval for the total weight of a seizure of illicit drugs present in a population. Their approach has subsequently been applied by several researchers in the field. The formula on which their approach is based does, however, not fully take into account the proportion of drug units found in the sample. In this paper, a modification is presented that consistently uses the correct sample size in all terms of the confidence interval, based on the proportion of drug units found in the sample. The effective sample size is smaller than the original sample size, and this should consequently be accounted for in the estimation of the standard error and in the corresponding t-distribution. The new confidence interval is again based on the assumption that the proportion of drug units in the population is known after sampling

Zheng F, Hu B*// *Wuhan Univ, Dept Chem, MoE Key Lab Anal Chem for Biol & Med, CN-430072 Wuhan, Peoples Rep China
J Mass Spectrom 2010 **45** (2) 205
Dual-column capillary microextraction (CME) combined with electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS) for the speciation of arsenic in human hair extracts
In this work, dual-column capillary microextraction (CME) system consisting of *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS)-silica coated capillary (C1) and 3-mercaptopropyl trimethoxysilane (MPTS)-silica coated capillary (C2) was developed for sequential separation/preconcentration of arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid [MMA(V)] and dimethylarsinic acid [DMA(V)] in the extracts of human hair followed by electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS) detection with iridium as permanent modifier. Various experimental parameters affecting the dual-column microextraction of different As species had been investigated in detail. It was found that at pH 9, As(V) and MMA could be quantitatively retained by C1 and only As(III) could be quantitatively retained by C2. With the aid of valve switching, As(V)/MMA(V) retained on C1 and As(III) retained on C2 could be sequentially desorbed by 10 µl of 0.01 mol/l HNO(3) [for As(V)], 0.1 mol/l HNO_3 [for MMA(V)] and 0.2 mol/l HNO_3-3% thiourea (m/v) [for As(III)], respectively, the eluents were immediately introduced into the Ir-coated graphite tubes for further ETV-ICP-MS detection. With two-step ETV pyrolysis program, Cl^- in the sample matrix could be *in situ* removed, and the total As in the human hair extracts or digested solution could be interference-free, determined by ETV-ICP-MS. DMA(V) in the human hair extracts was obtained by subtraction of total As in the human hair extracts from other three As species. Under the optimized conditions, the detection limits (3 σ) of the method were 3.9 pg/ml for As(III), 2.7 pg/ml for As(V), 2.6 pg/ml for MMA(V) and 124 pg/ml for total As with the relative standard deviations less than 7.0% (C = 0.1 ng/ml, *n* = 7), and the enrichment factor was 286, 262 and 260 for As(III), As(V) and MMA(V), respectively. The developed method was successfully applied for the speciation of arsenic in the extracts of human hair

13 Alcohol

Keten A, Tumer AR*, Balseven-Odabasi A// *Hacettepe Univ, Fac Med, Dept Forensic Med, Ankara, Turkey
Forensic Sci Int 2009 **193** (1-3) 101
Measurement of ethyl glucuronide in vitreous humor with liquid chromatography-mass spectrometry
Whereas it is important to detect alcohol consumption in postmortem investigations, it can be difficult to interpret the results of analyses of putrefied corpses. In order to mitigate this problem, studies have been undertaken to detect ethyl glucuronide (EtG), a non-oxidative metabolite of ethanol. An investigation was conducted of EtG levels in vitreous humor (VH), a useful tissue for postmortem investigations, and to compare VH EtG levels with blood and urine EtG levels. Liquid chromatography-mass spectrometry was used to investigate EtG in blood, urine and VH specimens from 25 cases. VH EtG was in measurable amounts in 19 cases. VH EtG levels ranged from 0.05 to 1.90 mg/l but were below the limit of detection (LOD is 0.03 mg/l) in six cases. Blood EtG was in measurable amounts in 21 cases. Blood EtG levels ranged from 0.64 to 5.82 mg/l but were below the limit of detection (LOD is 0.083 mg/l) in four cases. EtG was detected in urine in 17 cases (*n*: 19). Urine EtG levels ranged from 0.25 to 623 mg/l but were below the limit of detection (LOD is 0.12 mg/l) in one case. There was a significant correlation between VH EtG levels and those of blood and urine. Data indicate that postmortem VH EtG may be analysed to reveal alcohol intake

14 Tobacco

Adam T, McAughey J, Mocker C, McGrath C, Zimmermann R// Univ Augsburg, Inst Phys, DE-86159 Augsburg, Germany
Anal Chim Acta 2010 **657** (1) 36
Influence of filter ventilation on the chemical composition of cigarette mainstream smoke

Smoking behaviour, tobacco blend and a variety of cigarette design parameters affect total yields of cigarette smoke constituents. Filter ventilation which is the diluting of smoke by providing a zone of microscopic holes around the circumference of the filter is one method to reduce the yield of 'tar' and other smoke compounds. Little is known how these design variations influence the combustion conditions and consequently the overall chemical pattern of the smoke. Single photon ionization-time-of-flight mass spectrometry (SPI-TOFMS) was employed to analyse and compare cigarettes on a puff-by-puff basis, which differ only in filter ventilation magnitude. Cigarettes under investigation were made from Virginia tobacco and featured filter ventilations of 0% (no ventilation), 35%, and 70%. Cigarettes were smoked under two different puffing regimes. Puffing parameters of the conventional International Organization for Standardization (ISO) smoking regime or a more intense smoking condition were employed. Data indicate that each variation produces a change of the chemical pattern. In general, cigarettes with 0% filter ventilation as well as the intense smoking regime lead to a more complete combustion compared to the ISO smoking conditions and the high ventilated cigarettes. Changes in the overall patterns may also be demonstrated during the smoking for individual puffs. Some compounds predominate in the first puff, others are more pronounced in the middle puffs, whereas some are characteristic of the last puffs. This illustrates the high complexity of the occurring processes. Data might assist an understanding of the formation and decomposition of compounds taking place when a cigarette is smoked. In addition, it might facilitate targeted reduction strategies for specific toxicants or groups of toxicants in the smoke

Lafay F, Vulliet E*, Flament-Waton MM// *CNRS Service Central Anal - USR59, Chemin du Canal, FR-69360 Solaize, France

Anal Bioanal Chem 2010 **396** (2) 937

Contribution of microextraction in packed sorbent for the analysis of cotinine in human urine by GC-MS

Sample preparation by the relatively new procedure of microextraction in packed sorbent (MEPS) and analysis by GC-MS was employed to develop a simple, rapid, sensitive, and non-consuming solvent method for the determination of cotinine in urine. The optimized technique was compared with conventional solid-phase extraction/liquid-liquid extraction method used as reference. The wide linear range (5-5,000 ng/ml) and high sensitivity of the MEPS method (limit of detection 0.8 ng/ml) facilitates urinalysis of smokers as well as non-smokers susceptible to passive smoking

15 Homeland Security

Blome MC, Petro KA, Schengrund CL*// *Pennsylvania State Univ, Coll Med, Dept Biochem & Mol Biol, 500 University Dr, Hershey, Pa 17033, USA

Anal Biochem 2010 **396** (2) 212

Surface plasmon resonance analysis of ricin binding to plasma membranes isolated from NIH 3T3 cells

The potential for bioterrorism is an growing phenomenon. To mitigate the threat, the need for simple procedures to identify potential inhibitors of the binding of such biological agents to cell membranes has increased. Surface plasmon resonance (SPR) has been employed to monitor the binding of ricin, a ribosome-inactivating protein, to the plasma membranes of NIH 3T3 cells. The efficacy of the method to monitor the effectiveness of compounds at inhibiting ricin binding was determined by measuring the IC_{50} values for asialofetuin (ASF) and for bovine serum albumin derivatized with an average of 34 lactosyl moieties (BSA-Lac_{34}). Data suggest that SPR is an efficient technique for assaying the adherence of a toxin to isolated cell plasma membranes. In addition, SPR may indicate whether a compound that is an effective inhibitor of binding when a single ligand such as ASF is used will be equally effective when employed in studies with cells that express multiple cell surface ligands for ricin and/or the inhibitor

Dworzanski JP, Dickinson DN, Deshpande SV, Snyder AP, Eckenrode BA// Science Applications International Corporation, Aberdeen Proving Ground, Md 21010, USA

Anal Chem 2010 **82** (1) 145

Discrimination and phylogenomic classification of *Bacillus anthracis-cereus thuringiensis* strains based on LC-MS/MS analysis of whole cell protein digests

Modern taxonomy, diagnostics, and forensics of bacteria profit from procedures that supply data for genome-based classification and identification of strains. Unfortunately, complete genome sequencing remains costly, lengthy, and labor intensive. Consequently, other techniques are required to predict genomic relatedness among strains in an economical and timely manner. DNA-DNA hybridization and techniques based on genome fingerprinting or sequencing selected genes such as16S rDNA, gyrB, or rpoB are frequently employed as phylogenetic markers. However, analyses of complete genome sequences indicated that global measures of genome relatedness, such as the average genome conservation of shared genes, might facilitate better strain resolution and produce phylogenies compatible with relatedness revealed by traditional phylogenetic markers. Bacterial genomes are typically of high gene density. Consequently, the integration of mass spectrometry-based proteomic techniques with statistical methods for phylogenomic classification of bacterial strains was examined. A set of well characterized *Bacillus cereus* group strains isolated from poisoned food was employed to describe a procedure that relies on liquid chromatography-electrospray ionization-tandem mass spectrometry of tryptic peptides derived from whole cell digests. Peptides were identified and matched to a prototype database (DB) of reference bacteria with fully sequenced genomes to determine their phylogenetic profiles. The profiles were analysed to estimate genomic similarities with DB bacteria predicted by fractions of shared peptides (FSPs). FSPs acted as descriptors for each food isolate and were jointly analyzed using hierarchical cluster analysis procedures to reveal relatedness among investigated strains. The data indicated that phylogenomic classification of tested food isolates was in agreement with results from established genomic methods, thereby validating the procedure. This method might be employed as an alternative technique to predict relatedness among microbial genomes of *B. cereus* group members and potentially avoid the necessity for whole genome sequencing for phylogenomic typing of strains

Hoggard JC, Wahl JH, Synovec RE, Mong GM, Fragga CG*// *Pacific NW Natl Lab, 902 Battelle Blvd, Richland, Wa 99354, USA

Anal Chem 2010 **82** (2) 689

Impurity profiling of a chemical weapon precursor for possible forensic signatures by comprehensive two-dimensional gas chromatography/mass spectrometry and chemometrics

The feasibility is examined of using analytical and chemometric procedures to reveal and exploit the chemical impurity profiles from commercial dimethyl methylphosphonate (DMMP) samples to illustrate the type of forensic information that may be obtained from chemical-attack evidence. DMMP was employed as a model compound of a toxicant that may be deployed in a chemical attack. Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry (GC x GC/TOF-MS) was utilised to detect and identify trace organic impurities in six samples of commercially acquired DMMP. GC x GC/TOF-MS data were analyzed to illustrate impurity profiles for all six DMMP samples using 29 analyte impurities. PARAFAC was employed for the mathematical resolution of overlapped GC x GC peaks to ensure clean spectra for the identification of many of the detected compounds by spectral library matching. Statistical pairwise comparison demonstrated that there were trace impurities that were quantitatively similar/different among five of the six DMMP samples. Two of the DMMP samples were shown to have identical impurity profiles with this approach. The use of nonnegative matrix factorization suggested that there were five distinct DMMP sample types as demonstrated by the clustering of the multiple DMMP analyses into five distinct clusters in the scores plots. The two indistinguishable DMMP samples were confirmed by their chemical supplier to be from the same bulk source. Sample information from the other chemical suppliers supported the premise that the other four DMMP samples probably originated from different bulk sources. The data demonstrate that the matching of synthesized products from the same source is possible using impurity profiling. Furthermore, the identified impurities common to all six DMMP samples provide strong evidence that basic route information may be obtained from impurity profiles. Finally, impurities that may be unique to the sole bulk manufacturer of DMMP were found in some of the DMMP samples

Melchior WB, Tolleson WH// US/FDA, Div Biochem Toxicol, Natl Ctr Toxicol Res, Jefferson, Ar 72079, USA

Anal Biochem 2010 **396** (2) 204

A functional quantitative polymerase chain reaction assay for ricin, Shiga toxin, and related ribosome-inactivating proteins

Ricin, abrin, and other ribosome-inactivating proteins are potent toxins which deadenylate a specific base in 28S ribosomal RNA destroying ribosomes and resulting in cell death. Reverse transcriptase preferentially inserts an adenine opposite to an abasic site in RNA and this has enabled the development of a quantitative polymerase chain reaction (PCR) assay to detect the damage. The resulting assay detects as little as 30pg of ricin. The assay was employed to study the enzymatic properties of ricin such as pH and temperature optima (pH 4.5-5.0 and 60 °C)

Ovenden SPB, Gordon BR, Bagas CK, Muir B, Rochfort S, Bourne DJ// Defence Sci & Technol Organization, Fishermans Bend, Vic, Australia
Aust J Chem 2010 **63** (1) 8
A study of the metabolome of *Ricinus communis* for forensic applications
The appropriateness of the metabolome of *Ricinus communis* was studied in respect of cultivar and provenance determination. Seeds from 14 R. communis specimens (a total of 56 seeds) obtained from the east coast of Australia were analyzed by high pressure liquid chromatography with UV detection (HPLC-UV), liquid chromatography-mass spectrometry (LC-MS), and ^{1}H NMR spectroscopy. Collected data were analyzed using principle component analysis (PCA). For HPLC-UV analysis, six *R. communis* specimens were decisively identified by PCA as originating from separate classes relating to specimen. LC-MS data allowed unique ions to be identified for four specimens. By contrast, 10 specimens were precisely segregated with the PCA of the ^{1}H NMR data. The ratio of ricinine 1 to demethylricinine analogues 2 and 3 was found to be significant for specimen determination. Combined analysesindicate that a combination of HPLC-UV and ^{1}H NMR in conjunction with PCA might facilitate specimen differentiation

16 Workplace

Fernandez-Alvarez M, Lores M, Jover E, Garcia-Jares C, Bayona JM, Llompart M*// *Univ Santiago de Compostela, Inst Invest & Anal Alimentarios, Dept Quim Anal Nutr & Bromatol, ES-15782 Santiago de Compostela, Spain
J Chromatogr A 2009 **1216** (51) 8969
Photo-solid-phase microextraction of selected indoor air pollutants from office buildings. Identification of their photolysis intermediates
The photolysis of coumarin, butylated hydroxytoluene, 2,6-diisopropylnaphthalene, three common indoor air pollutants has been studied with photo-solid-phase microextraction (photo-SPME). Compounds were first extracted by SPME. Subsequently, the fibre was exposed to an irradiation source (i.e. xenon arc or low-pressure mercury lamp) for the selected time (from 2 to 120 min). Analyses of the irradiated fibres were performed with gas chromatography-mass spectrometry (GC-MS) detection. Photodecay kinetics showed first-order behaviour and the rate constants and half-life times were deduced. By means of their mass spectra, twenty-five photoproducts have been tentatively identified. On the basis of the identified transformation analytes, some photodegradation pathways are suggested. Photoformation-photodecay kinetics of identified by-products were also monitored by photo-SPME. It is believed that the photolytic routes of coumarin, butylated hydroxytoluene and 2,6-diisopropylnaphthalene have not been previously investigated

Tollback J, Isetun S, Colmsjo A, Nilsson U*// *Stockholm Univ, Dept Anal Chem, SE-10691 Stockholm, Sweden
Anal Bioanal Chem 2010 **396** (2) 839
Dynamic non-equilibrium SPME combined with GC, PICI, and ion trap MS for determination of organophosphate esters in air
A procedure for time-weighted average (TWA) air measurements of semivolatile organophosphate triesters, frequently employed as flame-retardants and plasticizers, and common indoor pollutants is presented. Dynamic non-equilibrium solid-phase microextraction (SPME) for air sampling when combined with GC/PICI and ion trap tandem MS, results in a fast, almost solvent-free procedure with low detection limits. Methanol was employed as reagent gas for PICI, resulting in stable protonated molecules and few fragments. A field sampler, in which a pumped airflow was applied over three polydimethylsiloxane (PDMS) 100-µm fibers in series was constructed, evaluated, and utilised for the measurements. The protocol LODs were in the range 2-26 ng/m for a sampling period of 2 h. Adsorption on SPME fibers was demonstrated to be about five times faster for triphenyl phosphate when compared with the other organophosphate esters under investigation, probably as a result of to more lipophilic properties of the aromatic compound. The boundary layer for triphenyl phosphate when employing a 100-µm PDMS sorbent was determined to 0.08 mm at a linear air velocity of 34 cm/s. Five different organophosphate triesters were revealed in air from a laboratory and a lecture hall, at concentrations ranging from 7 ng/m^3 up to 2.8 µg/m^3

17 Product Authenticity

Dolowy M, Niestroj A// Silesian Univ Med, Fac Pharm, Dept Anal Chem, 4 Jagiellonska St, PL-41200 Sosnowiec, Poland
J Liq Chromatogr Relat Technol 2010 **33** (1) 109
Densitometric determination of ursodeoxycholic acid in pharmaceutical formulations in form of tablets and capsules
Quantitative analysis of ursodeoxycholic acid in commercial drugs has been accomplished with a very simple and rapid TLC densitometric procedure. Methanol was employed to extract ursodeoxycholic acid (UDCA) from pharmaceutical formulations in the form of tablets and capsules. Chromatography was performed on glass plates precoated with silica gel 60 F(254) (E. Merck, Art. 1.05715) and with a mobile phase composed of a mixture of *n*-hexane-ethyl acetate-acetic acid in volume composition 22:22:5. Under these optimum conditions the RF value for ursodeoxycholic acid was equal to 0.48. Following visualization of spots with the use of 10% H_2SO_4 and heating to a temperature of 120 °C, the chromatograms were quantitatively scanned with a densitometer at maximum wavelength λ = 360 nm. The data indicate that routine quantity control of ursodeoxycholic acid may be achieved in selected pharmaceutical formulations with this procedure

Dumarey M, Sneyers R, Janssens W, Somers I, Vander Heyden Y*// *Vrije Univ Brussel, Laarbeeklaan 103, BE-1090 Brussels, Belgium
Anal Chim Acta 2009 **656** (1-2) 85
Drug impurity profiling: Method optimization on dissimilar chromatographic systems Part I: pH optimization of the aqueous phase
Dissimilar chromatographic systems may facilitate drug impurity profiling. Screening a new-drug impurity mixture on different systems enhances the chance that all impurities will be revealed. It also permits identification of a suitable system for further method development. Several strategies have been evaluated to determine the optimal pH (of the buffer used in the mobile phase) from the screening results. Four or five dissimilar stationary phases were screened at four pH values (between 2.5 and 9.4) to enhance analysis of the sample and to select one column for the further development. Different linear models (straight lines, 2nd and 3rd degree polynomials) produced from these experiments were examined for their ability to estimate the retention times (t_R) of the impurities at intermediate pH values. The predicted t_R values were employed to calculate minimal resolutions and eventually to select an optimal pH at which the highest minimal resolution had been forecast. None of the applied models was accurate enough to estimate correctly which peaks were worst separated at the indicated optimal pH. The best strategy (applying a second degree polynomial describing the t_R measured at 3 consecutive screening pH values) succeeded in indicating an optimal pH at which a good separation of the impurities was possible. However, the resulting separation quality was not or only slightly better than the best separation obtained during screening. Therefore, it is suggested that the most (time-) efficient procedure to develop an impurity profile of a new drug is to screen it on four or five dissimilar columns and at four different pH values. Then retain the best screening conditions (without making predictions for intermediate conditions) for further development of the organic modifier composition of the mobile phase, and occasionally the temperature and the gradient. This is particularly appropriate when the profiles have a complexity similar to those studied in this research

Fu Q, Shou MS, Chien D, Markovich R, Rustum AM// Schering Plough Corp, Global Qual Serv Anal Sci, 1011 Morris Ave, Union, NJ 07083, USA
J Pharm Biomed Anal 2010 **51** (3) 617
Development and validation of a stability-indicating RP-HPLC method for assay of betamethasone and estimation of its related compounds
One of the corticosteriod group is betamethasone (9α-fluoro-16β-methylprednisolone). It is routinely used as an anti-inflammatory agent and provides the starting material for the manufacture various esters. A procedure has been developed and validated which can separate and accurately quantitate low levels of 26 betamethasone related compounds by means of stability-indicating reverse-phase high performance liquid chromatography (RP-HPLC). To demonstrate the stability-indicating capability of the method adequate separation of all potential betamethasone related compounds from betamethasone was achieved. It was also employed to analyze compounds present in aged and stress degraded betamethasone samples. Chromatographic separation of betamethasone and its related compounds was accomplished by using a gradient elution at a flow rate of 1.0ml/min on a ACE 3 C18 column (150mmx4.6mm, 3µm particle size, 100A pore size) at 40 °C. Mobile phase A of the gradient was 0.1% methanesulfonic acid in aqueous solution and mobile phase B was a mixture of *tert*-butanol and 1,4-dioxane (7:93, v/v). UV detection at 254nm was utilised to monitor the analytes. For betamethasone 21-aldehyde, the QL and DL were 0.02% and 0.01% respectively. For betamethasone and the other betamethasone related compounds, the QL and DL were 0.05% and 0.02%. The precision of betamethasone assay was 0.6% and the accuracy of betamethasone assay ranged from 98.1% to 99.9%

Ghugare P, Dongre V, Karmuse P, Rana R, Singh D, Kumar A, Filmwala Z// St Xaviers Coll, Dept Chem, Nadkarni Sacasa Res Lab, IN-400001 Mumbai, India

J Pharm Biomed Anal 2010 **51** (3) 532

Solid state investigation and characterization of the polymorphic and pseudopolymorphic forms of indapamide

Indapamide, a diuretic drug generally used for the treatment of hypertension was examined by solid state investigation with polymorphic screening. Notable differences were observed in the solid state properties of crystals confirming the existence of a polymorphic and three pseudopolymorphic forms of indapamide. Detailed methods of preparation of the polymorphs and pseudopolymorphs are described. X-ray powder diffraction (XRPD), diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were performed for the characterization of different crystalline forms of indapamide. The stoichiometric ratio of solvents associated with the drug molecules in the pseudopolymorphic forms were calculated using TGA, nuclear magnetic resonance (NMR) spectroscopy and headspace gas chromatographic (HS-GC) analysis

Hashem HAA// Zagazig Univ, Fac Pharm, Dept Anal Chem, Zagazig, Egypt

Chromatographia 2010 **71** (1-2) 31

Chromatographic application on calixarene bonded stationary phases: A stability indicating method for simultaneous determination of paracetamol, caffeine and acetylsalicylic acid in excedrin tablets

A procedure is described for the simultaneous determination of paracetamol, caffeine and acetylsalicylic acid in tablet formulation by means of routine-LC which is simple, rapid and accurate.The technique demonstrates a new application for the calixarene stationary phases. The three pharmaceuticals were chromatographically separted on a Caltrex BIIE column (250 x 4 mm, 5 µm) using a binary mobile phase of 14% ACN and 86% 50 mM NaH_2PO_4 pH 3.0 at 1 ml/min flow rate. Detection was at 214 nm and separation was achieved in under 15 min. The approach was validated for efficiency, linearity, accuracy, precision, limit of detection and quantification, specificity, stability and robustness. Limits of detection were 4.88, 9.77 and 78.13 ng per 10 µl of their injected volumes, respectively. Recovery values of this procedure ranged from 94.63 to 101.85 and the reproducibility was within 3.88. In addition, the technique may be employed for the separation and determination of salicylic acid which is taken to be the most important degradation product of acetylsalicylic acid

Jain PS// RC Patel Coll Pharm, IN-425405 Shirpur, Dhule Dist, MS, India

J Chromatogr Sci 2010 **48** (1) 45

Stability-indicating HPTLC determination of ambroxol hydrochloride in bulk drug and pharmaceutical dosage form

The analysis of ambroxol hydrochloride both as a bulk drug and in formulations was accomplished by the development and validation of a simple, selective, precise, and stability-indicating high-performance thin-layer chromatographic (HPTLC) procedure. The technique utilised HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of methanol-triethylamine (4:6 v/v). The combination resulted in a compact spot for ambroxol hydrochloride (R_f value of 0.53 +/- 0.02). Densitometric analysis of ambroxol hydrochloride was performed in the absorbance mode at 254 nm. Linear regression analysis data for the calibration plots demonstrated a good linear relationship with r^2 = 0.9966 +/- 0.0013 in respect of peak area in the concentration range 100-1000 ng/spot. The mean value +/- standard deviation of slope and intercept were 164.85 +/- 0.72 and 1168.3 +/- 8.26 in respect of peak area. The procedure was validated for precision, recovery, and robustness. Limits of detection and quantitation were 10 and 30 ng/spot, respectively. Ambroxol hydrochloride was subjected to oxidation and thermal conditions and underwent degradation. This demonstrates that the drug is susceptible to oxidation and heat. Statistical analysis shows that the technique is repeatable, selective, and accurate. Stability testing of new chemical entities is an essential component of drug development of ambroxol hydrochloride and for its estimation in plasma and other biological fluids. Statistical analysis reveals that the procedure is repeatable and selective for the analysis of ambroxol hydrochloride as bulk drug and in pharmaceutical formulations. The HPTLC procedure may be applied for identification and quantitative determination of ambroxol hydrochloride in bulk drug and dosage forms. The work was to determine the purity of the drug available from the various sources by detecting the related impurities

Kachrimanis K, Rontogianni M, Malamataris S// Univ Thessaloniki, Sch Pharm, Dept Pharmaceut Technol, GR-54124 Thessaloniki, Greece

J Pharm Biomed Anal 2010 **51** (3) 512

Simultaneous quantitative analysis of mebendazole polymorphs A-C in powder mixtures by DRIFFS spectroscopy and ANN modeling

The simultaneous quantitative analysis of mebendazole polymorphs A-C in powder mixtures has been achieved by development of a protocol based on diffuse reflectance FTIR spectroscopy (DRIFTS) and artificial neural network (ANN) modeling. Spectral differences between the polymorphs were deduced by computationally assisted band assignments based on quantum chemical calculations, and subsequently, the spectra were preprocessed by calculation of 1st and 2nd derivatives. ANN models were then fitted after PCA compression of the input space. Finally the predictive performance of the ANNs was compared with that of PLS regression. It was noted that simultaneous quantitative analysis of forms A-C in powder mixtures was possible by fitting an ANN model to the 2nd derivative spectra even after PCA compression of the data (RMSEP of 1.75% for form A, 1.85% for B, and 1.65% for C), while PLS regression, applied for comparison purposes, resulted in acceptable predictions only within the 700-1750/cm spectral range and after direct orthogonal signal correction (DOSC), with RMSEP values of 2.69%, 2.68%, and 3.40% for forms A, B, and C, respectively. Application of the ANN to commercial samples of raw material and formulation (suspension) demonstrated its suitability for the analysis of polymorphic content

Kesting JR, Huang J, Sorensen D*// *Merck Frosst Canada Ltd, Merck Frosst Ctr Therapeut Res, 16711 Trans Canada Highway, Kirkland, Quebec, Canada H9H 3L1

J Pharm Biomed Anal 2010 **51** (3) 705

Identification of adulterants in a Chinese herbal medicine by LC-HRMS and LC-MS-SPE/NMR and comparative *in vivo* study with standards in a hypertensive rat model

Anecdotal evidence suggests an anti-hypertensive effect of Gold Nine Soft Capsules. Therefore, an *in vivo* study of this complex Chinese "herbal-based" medicine was undertaken. Treatment of spontaneous hypertensive rats with the contents of Gold Nine capsules indicated a notably positive effect. Therefore, an investigation was made to identify the active components. However, this led to the eventual identification of three known anti-hypertensive drugs (amlodipine, indapamide and valsartan) which were had not been declared on the label. Components were quickly identified using LC-HRMS and LC-MS-SPE/NMR and quantified by HPLC. The *in vivo* activity of a combination of commercially purchased standards was shown to be equivalent to that of the capsule content. Adulteration of herbal remedies and dietary supplements with synthetic drugs is increasingly common and may lead to serious adverse effects. LC-MS-SPE/NMR as a procedure for the rapid identification of such adulterants is exemplified in this case study

Kim SH, Lee J, Yoon T, Choi J, Choi D, Kim D, Kwon SW*// *Seoul Natl Univ, Coll Pharm, Seoul 151 742, South Korea

Biomed Chromatogr 2009 **23** (12) 1259

Simultaneous determination of anti-diabetes/anti-obesity drugs by LC/PDA, and targeted analysis of sibutramine analog in dietary supplements by LC/MS/MS

The safety of dietary supplements is questionable as there have been occasional reports of products contaminated with illegal adulterants. The present study was carried out to develop trustworthy methodologies to screen for six anti-diabetic drugs (phenformin, rosiglitazone, glipizide, glimepiride, glybenclamide and gliclazide) and six anti-obesity drugs (ephedrine, fenfluramine, T_3, T_4, fluoxetine and sibutramine) in dietary supplements. A simultaneous determination method of the 12 drugs by liquid chromatography coupled with a photodiode array (LC/PDA) was established and was validated for linearity (r^2 > 0.99), precision (RSD <13.3%), recoveries (88.8-115.9%) and reproducibility. Sibutramine and its analogs, *N*-desmethylsibutramine, were subject to further investigation by LC/MS/MS because they were one of the major illegal adulterants. Our proposed method to monitor illegal drug adulterations in dietary supplements using LC/PDA is a simple and reliable, and therefore applicable to routine drug-adulteration screening

Low MY, Zeng Y, Li L, Ge XW, Lee R, Bloodworth BC, Koh HL*// *Natl Univ Singapore, Fac Sci, Dept Pharm, Science Drive 4, SG-117543 Singapore, Rep Singapore

Drug Saf 2009 **32** (12) 1141

Safety and quality assessment of 175 illegal sexual enhancement products seized in red-light districts in Singapore

In order to enhance sexual performance, there has been increasing interest recently in the use of herbs and supplements as an alternative to drugs employed to treat erectile dysfunction. However, adverse events following the consumption of natural health products for sexual enhancement and the treatment of

erectile dysfunction have been reported. This research was conducted to assess the safety and quality of 175 sexual enhancement health products seized from makeshift stalls in red-light districts of Singapore. The Health Sciences Authority, Singapore, conducted 7 raids in two red-light districts in February and March 2008. A total of 175 sexual enhancement health products seized from makeshift stalls. They were extracted with methanol and screened for Western drug adulterants using high performance liquid chromatography and gas chromatography-mass spectrometry. The labels and claims of the products were also examined. Of the 175 products analysed, 134 (77%) contained adulterants of Western drugs or their analogues. In addition, 123 were found to contain with sildenafil. The scope of adulteration of these illegal health products with Western drugs, including synthetic phosphodiesterase type 5 enzyme (PDE-5) inhibitors and the risks of consuming such illegal sexual enhancement products are evaluated. Due to the extent of the raids, sildenafil was the most frequent adulterant discovered. Furthermore, some alternative products were found to contain high contents of sildenafil (>100 mg) plus high levels of the antidiabetic drug, glibenclamide (glyburide). Resultant severe hypoglycaemia has led to ten fatalities. Therefore, the unregulated presence of Western drug adulterants and their analogues in illegal sexual enhancement products seized from red-light districts in Singapore combined with frequently misleading labels and claims, places the health of consumers at risk. In respect of public health, greater public awareness of the danger of consuming such illegal products is necessary. In addition, the lack of quality control of these illegal sexual enhancement health products is crucial

Rao DD, Satyanarayana NV, Reddy AM, Sait SS, Chakole D, Mukkanti K// Dr Reddys Labs Ltd, Integrated Product Development Organisation, Bachupally Village, IN-500072 Hyderabad, India

J Pharm Biomed Anal 2010 **51** (3) 736

A validated stability-indicating UPLC method for desloratadine and its impurities in pharmaceutical dosage forms

A procedure has been developed for the determination of the purity of desloratadine in presence of its impurities and forced degradation products by means of a novel stability-indicating gradient reverse phase ultra-performance liquid chromatographic (RP-UPLC) technique. A procedure was developed employing a Waters Aquity BEH C18 column with mobile phase containing a gradient mixture of solvents A and B. Eluted compounds were monitored at 280nm. Run time was 8 min within which desloratadine and its five impurities were well separated. Desloratadine was degraded with oxidative, acid, base, hydrolytic, thermal and photolytic stress conditions. Desloratadine was noted to degrade significantly under oxidative and thermal stress but remain stable under acid, base, hydrolytic and photolytic conditions. Degradation products were well resolved from the main peak and its impurities confirming the stability-indicating power of the method. The developed procedure was validated as per ICH guidelines in respect of specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness. This procedure was also applicable for the assay determination of desloratadine in pharmaceutical dosage forms

Reddy PRM, Sreeramulu J, Naidu PY, Reddy AR// SK Univ, Dept Chem, IN-515003 Anantapur, India

Chromatographia 2010 **71** (1-2) 95

Stability indicating fast LC for the simultaneous estimation of intermediates and degradants of duloxetine hydrochloride

The simultaneous analysis of four impurities [(*S*)-*N*,*N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine, phenolic impurity, 1-napthol and duloxetine succinamide] of duloxetine hydrochloride, an antidepressant drug, has been accomplished by development and validation of a fast, selective and sensitive reversed phase liquid chromatographic procedure employing a C18 column. Successful separations were obtained by gradient elution with a mobile phase consisting of a mixture of phosphate buffer 14 mM containing 0.1 % of sodium octanesulfonate, pH 3.0, at a flow rate of 0.8 ml/min. The detection was at 220 nm. The technique was validated for precision, linearity and accuracy. The developed procedure was employed to quantify the impurities during stability sample analysis of duloxetine hydrochloride drug products

Shabir GA, Bradshaw TK, Arain SA, Shar GQ// Oxford Brookes Univ, Sch Life Sci, Oxford OX3 0BP, England

J Liq Chromatogr Relat Technol 2010 **33** (1) 61

A new validated method for the simultaneous determination of a series of eight barbiturates by RP-HPLC

The simultaneous determination of barbitone, allobarbitone, phenobarbitone, cyclobarbitone, hexobarbitone, pentobarbitone, secobarbitone and methohexitone compounds has been achieved in a single analytical run following development and validation of a new reversed-phase high performance liquid chromatographic (RP-HPLC) procedure. The technique employs a Phenosphere C18 (150 mm 4.6 mm; 5 µm) column and isocratic elution. The mobile phase consisted of a mixture of methanol-water (50:50, v/v), pumped at a flow rate of 1.0 ml/min. The UV detection is set at 254 nm. The procedure was validated in respect of accuracy, precision (repeatability and intermediate precision), specificity, linearity, range robustness and stability of analytical solutions. All the parameters investigated complied with the current recommendations for bioanalytical method validation. The technique is specific, simple, selective and reliable/ It may be routinely employed in quality control analysis of barbiturates raw materials for final product release

Soares R, Singh AK, Kedor-Hackmann ERM, Santoro MIRM*// *Univ Sao Paulo, Fac Pharmaceut Sci, Dept Pharm, CP 66083, BR-05315-970 Sao Paulo, Brazil

J AOAC Int 2009 **92** (6) 1663

Determination of atropine enantiomers in ophthalmic solutions by liquid chromatography using a Chiral AGP column

Many pharmaceuticals are commercialized under their racemic form. Enantiomers may exhibit differences in pharmacokinetic and pharmacodynamic profile. When a pure enantiomer in pharmaceutical formulations is employed there may be a better indication of therapeutic index and fewer adverse effects. Atropine is an alkaloid of *Atropa belladonna* and is a racemic mixture of *l*-hyoscyamine and *d*-hyoscyamine. It is widely employed to dilate the pupil. Quantification these enantiomers in ophthalmic solutions was achieved by development and validation of an HPLC technique employing a Chiral AGP column at 20 °C. The mobile phase consisted of a buffered phosphate solution (containing 10 mM 1-octanesulfonic acid sodium salt and 7.5 mM triethylamine, adjusted to pH 7.0 with orthophosphoric acid) and acetonitrile (99 + 1, v/v). The flow rate was 0.6 ml/min, with UV detection at 205 nm. In the concentration range of 14.0-26.0 µg/ml, the procedure was found to be linear (r > 0.9999), accurate (with recovery of 100.1-100.5%), and precise (RSD system < or = 0.6%; RSD intraday < or = 1.1%; RSD interday < or = 0.9%). The technique was specific. Standard and sample solutions were stable for up to 72 h. The factorial design guarantees robustness with a variation of +/- 10% in the mobile phase components and 2 °C of column temperature. Complete validation, including stress testing and factorial design, was examined and results presented

Sun CR, Wu JM, Wang DH, Pan YJ// Zhejiang Univ, Dept Chem, CN-310027 Hangzhou, Peoples Rep China

J Pharm Biomed Anal 2010 **51** (3) 778

Characterization of a novel impurity in bulk drug eprosartan by ESI/MS^n and NMR

A procedure suitable for eprosartan analysis was developed employing simple and sensitive liquid chromatography tandem multi-stage mass spectrometry (HPLC/MS^n) and with which an unknown impurity in bulk drug eprosartan was discovered. The fragmentation behavior of eprosartan and the impurity in negative mode was investigated. Two molecules of CO_2 released by the eprosartan precursor ion were noted, while four molecules of CO_2 were donated by the deprotonated molecular ion to the MS^3 product ions of the impurity. In addition, a characteristic fragmentation ion at *m/z* 335 was noted in both eprosartan and the impurity indicating that the impurity might have two eprosartan units. The unknown impurity was originally suggested to be an eprosartan dimer connected *via* a methylene unit at the thiophene moiety on the basis of the multi-stage mass spectrometric and exact mass evidences. It was ultimately identifed as 4,4'-(5,5'-(1*E*,1'*E*)-3,3'-(4,4'-methylenebis(thiophene-4,2-diyl))bis(2-carboxyprop-1-ene-3,1-diyl)bis(2-butyl-1*H*-imidazole-5,1-diyl))bis(methylene) dibenzoic acid by NMR experiments including 1D (^{1}H NMR, ^{13}C NMR, DEPT135^0) and 2D (^{1}H-^{1}HCOSY, HMQC and HMBC) data

Suntornsuk L, Ployngam S// Mahidol Univ, Fac Pharm, Dept Pharmaceut Chem, 447 Sri Ayudhaya Rajathevee, TH-10400 Bangkok, Thailand

J Pharm Biomed Anal 2010 **51** (3) 541

Simultaneous determination of *R*-(-)-, *S*-(+)-baclofen and impurity A by electrokinetic chromatography

A rapid technique for the simultaneous analysis of *R*-(-)-, *S*-(+)-baclofen and impurity A, (4*RS*)-4-(4-chlorophenyl) pyrrolidin-2-one, by electrokinetic chromatography has been developed. The optimized condition was in 100mM sodium borate buffer (pH 9.9) containing 18mM α-cyclodextrin (CD) and 1% (v/v) ACN using a fused-silica capillary dynamically coated with polyethylene oxide (PEO), with an effective length of 56cm and an inner diameter of 50µm, hydrodynamic injection at 50mbar for 6s, temperature of 45 °C, applied voltage of 27kV and UV detection at 220nm. Baseline separation of all analytes was accomplished within 9min (R_s > 2.7) with the migration order of impurity A, *S*-(+)- and *R*-(-)-baclofen. The procedure exhibited good linearity (r^2>0.999 in a range of 5-50µg/ml for impurity A and 50-500µg/m/ for baclofen

enantiomers), precision (%RSDs<3.37%) and recoveries (100.3% for *R*-(-)-baclofen, 101.6% for *S*-(+)-baclofen and 96.1% for impurity A). Detection and quantitation limits were 10 and 30µg/ml for both enantiomers, and 2 and 5µg/ml for the impurity, respectively. The protocol was efficient for the analysis of baclofen enantiomers and impurity A in pharmaceutical raw material and formulations because to its reliability, speed and simplicity

Swami K, Judd CD, Orsini J// Wadsworth Ctr, New York State Dept Hlth, Lab Inorgan & Nucl Chem, Albany, NY 12201, USA
Spectrosc Lett 2009 **42** (8) 479
Trace metals analysis of legal and counterfeit cigarette tobacco samples using inductively coupled plasma mass spectrometry and cold vapor atomic absorption spectrometry
The determination of trace amounts of Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, Tl, and Pb by inductively coupled plasma mass spectrometry and Hg by cold-vapor atomic absorption spectrometry in cigarette tobacco samples was achieved following development of a closed-vessel microwave-digestion procedure. In order to assess the effectiveness of the digestion process, recovery studies were conducted using solutions prepared from National Institute of Standards and Technology Standard Reference Material 1573a Trace Elements in Tomato Leaves and Polish Certified Reference Material Virginia Tobacco Leaves. Limits of detection were below 1 µg/g for all elements studied. Samples from two genuine-brand and three counterfeit packs were analyzed. The mean amounts of Be, As, Mo, Cd, Sb, Tl, Pb, and Hg were greater in counterfeit cigarettes. However, the amounts of V, Cr, Mn, Co, Cu, Zn, Se, and Ba were comparable among legal and counterfeit cigarettes. The amount of Ni on-the-other-hand was greater in the legal cigarettes. Evaluation of Be, As, Mo, Cd, Sb, Tl, Pb, and Hg with their potential hazards for smokers is briefly discussed

Trefi S, Gilard V, Balayssac S, Malet-Martino M*, Martino R// *Univ Paul Sabatier, CNRS UMR 5068, Lab SPCMIB, Grp RMN Biomed, 118 Route Narbonne, FR-31062 Toulouse, France
Magn Reson Chem 2009 **47** (Spec Iss 1) S163
The usefulness of 2D DOSY and 3D DOSY-COSY ^{1}H NMR for mixture analysis: Application to genuine and fake formulations of sildenafil (Viagra)
Two-dimensional diffusion ordered spectroscopy (DOSY) ^{1}H NMR is proposed to analyze drugs that are complex mixtures in order to discriminate genuine from fake formulations. The method was applied to the analysis of 17 formulations of sildenafil, one being genuine Viagra and the others illegally manufactured formulations of this drug coming from India, Syria and China. It enabled (i) distinguishing imitations or counterfeit from the authentic formulation, (ii) detecting the presence of sildenafil or adulterants, (iii) gaining information on the formulation process by detection of various excipients, thus giving a precise and global 'signature' of the manufacturer. Even though some samples are slightly overdosed, the quality of products manufactured in India and Syria was better than that of Chinese formulations which were adulterated with vardenafil and homosildenafil. This study also presents a three-dimensional DOSY-COSY ^{1}H NMR experiment that provides both virtual separation and structural information

Venhuis BJ, Zomer G, Vredenbregt MJ, De Kaste D// Natl Inst Publ Hlth & Environm, Postbox 1, NL-3721 MA Bilthoven, The Netherlands
J Pharm Biomed Anal 2010 **51** (3) 723
The identification of (-)-*trans*-tadalafil, tadalafil, and sildenafil in counterfeit Cialis and the optical purity of tadalafil stereoisomers
Four blisters of suspect Cialis (tadalafil) 20mg tablets were screened for authenticity using near infrared spectroscopy (NIRS). They were also tested for the presence of phosphodiesterase 5 (PDE-5) inhibitors using LC-DAD-MS. All suspect samples were identified as counterfeit Cialis. They contained sildenafil or tadalafil and sildenafil together. Whereas the samples contained efficacious amounts of PDE-5 inhibitors, neither the active ingredient nor the dosage corresponded with the product description on the pack.This is the first reported instance of a diastereomeric mixture of tadalafil and *trans*-tadalafil (3:1) being identified in a counterfeit medicine. LC-DAD-CD revealed that both diastereomers had a high optical purity. The optical rotation of the diastereomeric mixture was noted and indicated the presence of (-)-*trans*-tadalafil, which is the only other stereoisomer with some PDE-5 inhibitory activity. There are no safety profiles are known for the stereoisomers of tadalafil and thus there is a potential health risk. Furthermore, the optical purity of tadalafil requires consideration when calculating the dosage in illegal medicines

Wu LM, Zhou L, Vogt FG, Liu DQ// GlaxoSmithKline Inc, Anal Sci, Chem Dev, 709 Swedeland Rd, King of Prussia, Pa 19406, USA
J Pharm Biomed Anal 2010 **51** (3) 577
Gas-phase Smiles rearrangement in structural analysis of a pseudo-oxidative impurity generated in the pharmaceutical synthesis of *S*-(thiobenzoyl)thioglycolic acid
Several mass spectrometry (MS) procedures including accurate MS and MS/MS, plus hydrogen/deuterium (H/D) exchange, were employed to characterize a pseudo-oxidative reaction by-product (impurity I) in the pharmaceutical synthesis of *S*-(thiobenzoyl)thioglycolic acid. The negative ion MS/MS data produced complementary structural information to the positive ion MS/MS data. An insight to the gas-phase Smiles rearrangement upon collision-induced dissociation (CID) in the negative ion MS/MS mode was important to the structural elucidation of impurity I. Theoretical calculations by density functional theory (DFT) at the B3LYP/6-311G(d,p) level resulted in an understanding of the thermochemistry of the Smiles rearrangement reaction. It is suggested that the pseudo-oxidative impurity is generated *via* the base-catalyzed hydrolysis in solution

Zhang ZQ, Li BYZ, Suwan J, Zhang FM, Wang ZY, Liu HY, Mulloy B, Linhardt RJ*// *Rensselaer Polytech Inst, Dept Chem & Chem Biol, Troy, NY 12180, USA
J Pharm Sci 2009 **98** (11) 4017
Analysis of pharmaceutical heparins and potential contaminants using ^{1}H-NMR and PAGE
In 2008, heparin (active pharmaceutical ingredient, API) lots were associated with anaphylactoid-type reactions. Oversulfated chondroitin sulfate (OSCS), a semi-synthetic glycosaminoglycan (GAG), was identified as a contaminant and dermatan sulfate (DS) as an impurity. While DS has no known toxicity, OSCS was toxic leading to patient deaths. Heparins, prepared before these adverse reactions, needed to be screened for impurities and contaminants. Heparins were analyzed using high-field ^{1}H-NMR spectroscopy. Heparinoids were mixed with a pure heparin and analyzed by ^{1}H-NMR to assess the utility of ^{1}H-NMR for screening heparin adulterants. Sensitivity of heparinoids to deaminative cleavage, a method widely used to depolymerize heparin, was evaluated with polyacrylamide gel electrophoresis to detect impurities and contaminants, giving limits of detection (LOD) ranging from 0.1% to 5%. Most pharmaceutical heparins prepared between 1941 and 2008 showed no impurities or contaminants. Some contained DS, CS, and sodium acetate impurities. Heparin prepared in 2008 contained OSCS contaminant. Heparin adulterated with heparinoids showed additional peaks in their high-field ^{1}H-NMR spectra, clearly supporting NMR for monitoring of heparin API with an LOD of 0.5-10%. Most of these heparinoids were stable to nitrous acid treatment suggesting its utility for evaluating impurities and contaminants in heparin API

18 Techniques

Abu-Rabie P, Spooner N// GlaxoSmithKline R&D, Preclinical Dev Drug Metab & Pharmacokinetics, Park Rd, Ware SG12 0DP, England
Anal Chem 2009 **81** (24) 10275
Direct quantitative bioanalysis of drugs in dried blood spot samples using a thin-layer chromatography mass spectrometer interface
CAMAG thin-layer chromatography mass spectrometer (TLC-MS) interface has been tested as an instrument for the direct quantitative bioanalysis of drugs from dried blood spot (DBS) samples, employing an MS detector, plus/minus high-performance liquid chromatography (HPLC) separation. The method resulted in acceptable sensitivity, linearity, accuracy, and precision data for bioanalytical validations with and without the inclusion of HPLC separation. Furthermore, the direct elution technique was demonstrated to increase assay sensitivity for a range of analytes representing a wide "chemical space" for pharmaceutical-type molecules greater than that produced by conventional manual extraction of samples (punching of DBS and elution with solvent prior to HPLC-MS analysis). Procedures were carried out to optimize extraction time, minimize sample-to-sample carry-over, and compare chromatographic performance. Data from this this preliminary study indicate that the TLC-MS interface has the potential to be an effective instrument for the direct analysis of drugs in DBS samples at relevant concentrations. This protocol might result in significant time and cost savings and greatly simplify bioanalytical procedures compared with current manual procedures. In addition, the greater sensitivity compared with that of manual extraction might facilitate the analysis of compounds not currently amenable to DBS sampling due to limitations in assay sensitivity

Al Azzam KM, Saad B, Aboul-Enein HY*, Elbashir AA// *Natl Res Ctr, Pharmaceut & Med Chem Dept, Tahrir St, EG-12311 Cairo, Egypt
J Liq Chromatogr Relat Technol 2010 **33** (2) 167

Assay and stability-indicating capillary zone electrophoretic method for the determination of modafinil in bulk and its pharmaceutical preparations

Modafinil is a stimulant employed pharmaceutical formulations. A simple, sensitive, and cost effective capillary zone electrophoresis (CZE) procedure for its determination has been developed and validated. CZE separation was performed using 50 µm i.d x 56 cm fused silica capillary. Temperature was 25 °C. Applied voltage was 25 kV. 20 mM H_3PO_4 - 1 M tris as running buffer (pH 9.0). Detection wavelength, 225 nm. Phenobarbital was employed as the internal standard. The technique was validated and showed both good precision and accuracy and also good robustness. The calibration was linear from 5 to 250 µg/ml. Accuracy values ranged from 101.6 to 105.3%. The good accuracy values produced suggest the potential of this procedure for the determination of the analyte in pharmaceutical formulations. The LOD and LOQ were 1.2 and 3.5 µg/ml, respectively. The technique was successfully employed in the determination modafinil in pharmaceutical tablet formulations. Excipients present in the tablets and degraded products as a result of different stress conditions did not interfere in the assay

Ali I, Saleem K, Gaitonde VD, Aboul-Enein HY*, Hussain I// *Natl Res Ctr, Pharmaceut & Med Chem Dept, Pharmaceut & Drug Ind Res Div, EG-12311 Cairo, Egypt

Chirality 2010 **22** (1) 24

Chiral separations of some -adrenergic agonists and antagonists on AmyCoat column by HPLC

Sixteen β-adrenergic antagonists namely acebutalol, alprenolol, atenolol, bisoprolol, bopindolol, bufurolol, carazolol, celiprolol, indenolol, metaprolol, nebivolol, oxprenolol, practolol, propranolol, tertalol, and timolol, and two β-adrenergic agonists namely cimeterol and clenbuterol were resolved on AmyCoat (150 x 46 mm, 3 µm size of silica particle) by using (85:15:0.1, v/v/v), (90:10:0.1, v/v/v), and (95:05:0.1, v/v/v) combinations of *n*-heptane, ethanol, and diethylamine solvents, respectively. The flow rates used were 0.5, 1.0, 2.0, and 3.0 ml/min with detection at 225 nm. The values of capacity, separation, and resolution factors ranged from 0.38 to 19.70, 1.08-2.33, and 1.0 and 4.50, respectively. The maximum and minimum resolutions were achieved for celiprolol and bufurolol, respectively. The chiral recognition mechanisms were also discussed. The values of validation parameters were calculated.

Belal T, Awad T, Clark CR// Univ Alexandria, Fac Pharm, Dept Pharmaceut Anal Chem, EG-21521 Alexandria, Egypt

J AOAC Int 2009 **92** (6) 1622

Stability-indicating simultaneous determination of paracetamol and three of its related substances using a direct GC/MS method

The simultaneous determination of paracetamol (PR) and three of its related substances [4-aminophenol (4-AP), acetanilide (AD), and 4'-chloroacetanilide (4-CA)] was achieved by development of a simple, direct, and selective stability-indicating GC/MS technique. The procedure involves resolution of the underivatized compounds using a 100% dimethylpolysiloxane (Rtx-1) column, and MS detection was accomplished in the electron-impact mode. The four compounds were totally resolved in less than 11 min. Fragmentation pathways for the four compounds was determined and the structures of the major fragment ions peaks proposed. Quantification of the analytes was based on measuring their peak areas. Reliability and analytical performance of the proposed technique including linearity, range, precision, accuracy, and detection and quantification limits were statistically validated. Calibration curves were linear over the ranges 75-500, 25-350, 25-350, and 25-350 µg/ml for PR, 4-AP, AD, and 4-CA, respectively. The proposed technique was successfully employed for the analysis of PR and its related substances in laboratory-prepared mixtures of different proportions. In addition, the procedure was utilised for the assay of PR in several commercially available pharmaceutical formulations with recoveries of 98.95-100.76%

Bhushan R, Tanwar S// Indian Inst Technol, Dept Chem, IN-247667 Roorkee, India

Biomed Chromatogr 2009 **23** (12) 1291

Reversed-phase high-performance liquid chromatographic enantioresolution of six -blockers using dinitrophenyl-L-Pro-*N*-hydroxysuccinimide ester, *N*-succinimidyl-(*S*)-2-(6-methoxynaphth-2-yl) propionate and twelve variants of Sangar's reagent as chiral derivatizing reagents

Twelve chiral derivatizing reagents (CDRs) were synthesized by substituting one of the fluorine atoms in 1,5-difluoro-2,4-dinitrobenzene (DFDNB) with three optically pure amines [(*R*)-(-)-1-cyclohexylethylamine, (+)-dehydroabietylamine and (*S*)-(-)-α,4-dimethylbenzylamine], six amino acid amides [L-Ala-NH_2, L-Phe-NH_2, L-Val-NH_2, L-Leu-NH_2, L-Met-NH_2 and D-Phg-NH_2] and three amino acids [L-Ala, L-Val and L-Leu]. In addition, dinitrophenyl-L-Pro-*N*-hydroxysuccinimide ester and *N*-succinimidyl-(*S*)-2-(6-methoxynaphth-2-yl) propionate were also synthesized and used as CDR. Keeping in view the presence of an amino group, diastereomers of six β-blockers (atenolol, propranolol, bisoprolol, metoprolol, salbutamol, and carvedilol) were synthesized by reaction with these 14 CDRs. The diastereomers were separated by RP-HPLC. The method was validated for linearity, accuracy, limit of detection and limit of quantification

Cirilli R, Fiore S, La Torre F, Maccioni E, Secci D, Sanna NL, Faggi C// Ist Super Sanita, Dipt Farmaco, Viale Regina Elena 299, IT-00161 Rome, Italy

Chirality 2010 **22** (1) 56

Semipreparative HPLC enantioseparation, chiroptical properties, and absolute configuration of two novel cyclooxygenase-2 inhibitors

A direct semipreparative HPLC enantioseparation of two chiral thiazolidinone derivatives having cyclooxygenase-2 inhibition activity was performed on the Chiralpak IA chiral stationary phase. Semipreparative amounts of enantiopure forms were collected using acetonitrile-ethanol-trifluoroacetic acid mixtures as mobile phase. The absolute configuration of both compounds was unequivocally established by single-crystal X-ray diffraction method and correlated to the chiroptical properties of isolated enantiomers

Garcia-Garcia MA, Dominguez-Renedo O, Alonso-Lomillo A, Arcos-Martinez MJ*// *Univ Burgos, Fac Ciencias, Dept Quim, Area Quim Anal, Plaza Misael Banuelos s/n, ES-09001 Burgos, Spain

Sensor Lett 2009 **7** (4) 586

Electrochemical methods of carbamazepine determination

Glassy carbon (GCE), screen-printed carbon (SPCE) and silver nanoparticle-modified carbon screen-printed (AgNPs/SPCE) electrodes have been tested for the analysis of carbamazepine (CBZ) by employing the oxidation signal produced from differential-pulse adsorptive stripping voltammetry (DPAdSV). In addition, amperometric analysis of CBZ was carried out an electrochemical biosensor based on the immobilization of the enzyme horseradish peroxidase (HRP) on a SPCE. The best detection limit (0.938 =/- 0.006 µM) was achieved with the AgNPs/SPCE. However, the best selectivity was produced with the HRP based electrode. CBZ in pharmaceutical preparations were successfully analysed by the AgNPs/SPCE and the HRP-based SPCE developed in this study. Interferences resulting from other antiepileptic pharmaceuticals as phenytoin and phenobarbital on CBZ measurement were also investigated.

Gasparini M, Curatolo M, Assini W, Bozzoni E, Tognoli N, Dusi G// Ist Zooprofilatt Sperimentale Lombardia & Emilia, Via Bianchi 9, IT-25124 Brescia, Italy

J Chromatogr A 2009 **1216** (46) 8059

Confirmatory method for the determination of nandrolone and trenbolone in urine samples using immunoaffinity cleanup and liquid chromatography-tandem mass spectrometry

Analysis of urine samples by liquid chromatography electrospray mass spectrometry (LC-MS-MS) has been developed for the simultaneous determination of nandrolone (α and β) and trenbolone (α and β). Following enzymatic deconjugation, urine was processed with a one-step cleanup on a commercially available immunoaffinity chromatography cartridge. Analysis was performed with liquid chromatography-positive ion electrospray tandem mass spectrometry using deuterium labelled internal standards. The technique was applicable to bovine and swine urine samples. The procedure was validated as a quantitative confirmatory method in accordance with the Commission Decision 2002/657/EC criteria. Data indicate that the protocol was suitable for statutory residues testing regarding the following performance characteristics: instrumental linearity, specificity, precision (repeatability and intra-laboratory reproducibility), recovery, decision limit (CCα), detection capability (CCβ) and ruggedness. Decision limits (CCα) were between 0.54 and 0.60 µg/l; the recovery was above 64% for all the analytes. Repeatability was between 1.6% and 5.7% and within-laboratory reproducibility between 1.6% and 6.0% for all the steroids

Gyllenhaal O, Karlsson A// Uppsala Univ, Fac Med, Div Anal Pharmaceut Chem, Biomed Ctr, Box 574, SE-75123 Uppsala, Sweden

Chromatographia 2010 **71** (1-2) 7

Evaluation conditions for SFC of metoprolol and related amino alcohols on Hypercarb (porous graphitic carbon) with respect to structure-selectivity relations

Mobile phase chromatographic conditions employing Hypercarb as support and carbon dioxide with addition of methanol as mobile phase have been examined

for the selective separation of metoprolol from related amino alcohols. In the current research, the unique ability of the porous graphitized carbon surface to separate closely structurally related substances was tested. Hypercarb and "chromatographic normal phase conditions" (SFC) were investigated in respect of how to control retention and to improve peak performance. A high concentration of basic aliphatic amine additive was necessary to facilitate the elution of the amine analytes as symmetrical peaks. *N,N*-Dimethyloctylamine performed better than triethylamine because the retention was noticeably shorter but the selectivity and resolution were almost the same. By adjusting the temperature of the column, the selectivity could be optimized. High selectivity was established between metoprolol and two homologues with one and two extra methylene groups inserted between the secondary nitrogen atom and the carbon atom with a hydroxyl group attached. The mobile phase flow rate altered the column efficiency only to a minor extent because the steepness of the van Deemter curve for metoprolol was almost flat in the range studied: 0.5-3.0 ml/min

Hashem H, Trundelberg C, Jira T*// *Ernst Moritz Arndt Univ Greifswald, Inst Pharm Pharmaceut Med Chem, F-L-Jahn Str 17, DE-17487 Greifswald, Germany

Chromatographia 2010 **71** (1-2) 91

Chromatographic application on calixarene bonded stationary phases: A stability indicating LC-method for determination of celecoxib in tablet formulation

The extraction and quantification of celecoxib in tablets has been accomplished by means of centrifugation of the fine powder of the tablets in acetonitrile (ACN). The extract was analysed by LC. Chromatographic separation was performed on a Caltrex Alll column, a relatively new packing material consisting of silica-bonded calix[8]-arene, using isocratic binary mobile phase of ACN and H_2O (55%:45%, v/v). A diode array detector was employed at 254 nm for detection. The procedure was validated for system suitability, linearity, precision, limits of detection and quantitation, specificity, stability and robustness. Limits of detection and quantitation were 0.122 and 0.488 µg/ml, respectively. The recovery value of this method was 101.88% and the reproducibility was within 2.08

Huang H, Jin JY, Hong JH, Kang JS*, Lee W// *Chungnam Natl Univ, Coll Pharm, Taejon 305 764, South Korea

Bull Korean Chem Soc 2009 **30** (11) 2827

Liquid chromatographic enantiomer separation of non-steroidal anti-inflammatory drugs on immobilized polysaccharide derived chiral stationary phase under reversed and normal phase mode

No abstract available

Joseph A, Rustum A// Schering Plough Corp, Global Qual Serv Anal Sci, 1011 Morris Ave, Union, NJ 07083, USA

J Pharm Biomed Anal 2010 **51** (3) 521

Development and validation of a RP-HPLC method for the determination of gentamicin sulfate and its related substances in a pharmaceutical cream using a short pentafluorophenyl column and a charged aerosol detector

Gentamicin sulfate is an effective broad spectrum aminoglycoside antibiotic employed as an active pharmaceutical ingredient (API) against both Gram-positive and Gram-negative bacteria. A reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed and validated to analyze the composition of gentamicin sulfate and to estimate its related substances (without any pre- or post-column derivatization) in a pharmaceutical cream. Gentamicin has a weak UV chromophore. Therefore, it is not possible to detect low levels of known and unknown related substances of gentamicin using a UV detector. With the current procedure, a charged aerosol detector (CAD) was employed to obtain the necessary high sensitivity. This technique facilitates the separation all the analogues of gentamicin including all known and unknown related substances of the API. A short (5cmx4.6mm) pentafluorophenyl HPLC column from Restek (Allure PFP) was employed with an ion-pair gradient mobile phase consisting of (A) heptafluorobutyric acid:water:acetonitrile (0.025:95:5, v/v/v) and (B) trifluoroacetic acid:water: acetonitrile (1:95:5, v/v/v)

Kamble AY, Mahadik MV, Khatal LD, Dhaneshwar SR*// *Bharati Vidyapeeth Univ, Dept Pharmaceut Chem, Poona Coll Pharm, IN-411038 Pune, India

Anal Lett 2010 **43** (2) 251

Validated HPLC and HPTLC method for simultaneous quantitation of amlodipine besylate and olmesartan medoxomil in bulk drug and formulation

The simultaneous analysis of amlodipine besylate and olmesartan medoxomil in formulation was achieved with two methods. The first technique employed HPTLC on Merck HPTLC aluminium sheets of silica gel 60 F254 using n-butanol: acetic acid: water (5:1:0.1, vlvlv) as the mobile phase. The second procedure utilised HPLC on a RP-PerfectSil-100 ODS-3-C18 column from MZ-Analysetechnik GmbH, Germany and acetonitrile/0.03 M ammonium acetate buffer (pH = 3) in a ratio of 55:45 as the mobile phase. Both protocols were been applied to formulation without interference of excipients of formulation

Nemet Z, Sajo I, Demeter A// Gedeon Richter Plc, Drug Polymorphism Res Div, POB 27, HU-1475 Budapest, Hungary

J Pharm Biomed Anal 2010 **51** (3) 572

Rietveld refinement in the routine quantitative analysis of famotidine polymorphs

The quantitative determination of famotidine polymorphic forms in their binary mixtures was achieved by development of an accurate, precise and reliable X-ray powder diffraction technique. This procedure is a small improvement on the previously established Raman method. This study demonstrates the advantage of focused beam transmission geometry in reducing the effect of preferred orientation in general, and the straightforward transmission foil sample preparation technique in facilitating high-throughput measurements in particular. This combination provides good quality data for Rietveld refinement which assures more reliable quantitative results than employing intensity ratios of selected single reflections. Following careful adjustment of profile parameters, simple routine application of the technique was accomplished

Oliveira PR, Bernardi LS, Mendes C, Sangoi MS, Silva MAS// Univ Fed Santa Catarina Hlth Sci Ctr, Dept Pharmaceut Sci, BR-88040-900 Florianopolis, SC, Brazil

J Pharm Biomed Anal 2010 **51** (3) 728

Liquid chromatographic determination of lumiracoxib in pharmaceutical formulations

The determination of lumiracoxib in pharmaceutical formulations was conducted using a stability-indicating reversed-phase liquid chromatography (LC) procedure which was both developed and validated. The LC method was carried out on a Synergi fusion C_{18} column (150 mm x 4.6mm) maintained at 30 °C. The mobile phase was composed of phosphoric acid (25 mM; pH 3.0)/acetonitrile (40:60, v/v), run at a flow rate of 1.0ml/min, and detection at 272nm. The chromatographic separation was obtained within 10 min and it was linear in the concentration range of 10-100 µg/ml (r^2 = 0.9999). Validation parameters such as the specificity, linearity, precision, accuracy, and robustness were ascertained and produced results within the acceptable range. Stress studies were conducted but no interference by the degradation products was noted. Furthermore, the proposed technique was successfully employed for the assay of lumiracoxib in pharmaceutical formulations

Oliveira-Silva D, Oliveira CH, Mendes GD, Galvinas PAR, Barrientos-Astigarraga RE, De Nucci G// USP, Av Lineu Prestes 748, Sala 970, Cidade Universitaria, Butanta, BR-05508-900 Sao Paulo, Brazil

Biomed Chromatogr 2009 **23** (12) 1266

Quantification of chlordesmethyldiazepam by liquid chromatography-tandem mass spectrometry: Application to a cloxazolam bioequivalence study

A rapid, sensitive and specific LC-MS/MS method was developed and validated for quantifying chlordesmethyldiazepam (CDDZ or delorazepam), the active metabolite of cloxazolam, in human plasma. In the analytical assay, bromazepam (internal standard) and CDDZ were extracted using a liquid-liquid extraction (diethyl-ether/hexane, 80/20, v/v) procedure. The LC-MS/MS method on a RP-C18 column had an overall run time of 5.0 min and was linear (1/x weighted) over the range 0.5-50 ng/ml (R > 0.999). The between-run precision was 8.0% (1.5 ng/ml), 7.6% (9 ng/ml), 7.4% (40 ng/ml), and 10.9% at the low limit of quantification-LLOQ (0.500 ng/ml). The between-run accuracies were 0.1, -1.5, -2.7 and 8.7% for the above mentioned concentrations, respectively. All current bioanalytical method validation requirements (FDA and ANVISA) were achieved and it was applied to the bioequivalence study (Cloxazolam - test, Eurofarma Lab. Ltda and Olcadil - reference, Novartis Biociencias S/A). The relative bioavailability between both formulations was assessed by calculating individual test/reference ratios for Cmax, AUClast and AUC0-inf. The pharmacokinetic profiles indicated bioequivalence since all ratios were as proposed by FDA and ANVISA

Pataj Z, Ilisz I, Berkecz R, Forro E, Fulop F, Peter A*// *Univ Szeged, Dept Inorgan & Anal Chem, Dom ter 7, HU-6720 Szeged, Hungary

Chirality 2010 **22** (1) 120

Comparison of separation performances of amylose- and cellulose-based stationary phases in the high-performance liquid chromatographic enantioseparation of stereoisomers of -lactams

High-performance liquid chromatographic methods were developed for the

separation of the enantiomers of 19 β-lactams. The direct separations were performed on chiral stationary phases containing either amylose-tris-3,5-dimethylphenyl carbamate, (Kromasil AmyCoat column) or cellulose-tris-3,5-dimethylphenyl carbamate, (Kromasil CelluCoat column) as chiral selector. The different methods were compared in systematic chromatographic examinations. The separations were carried out with good selectivity and resolution. The AmyCoat and CelluCoat columns appear to be highly complementary. The best separations of bi- and tricyclic β-lactam stereoisomers were obtained with the AmyCoat column, whereas the 4-aryl-substituted β-lactams were better separated on the CelluCoat column. The elution sequence was determined in all cases; no general rule could be established

See KL, Elbashir AA, Saad B, Ali ASM, Aboul-Enein HY*// *Natl Res Ctr, Pharmaceut & Med Chem Dept, EG-12311 Cairo, Egypt
Biomed Chromatogr 2009 **23** (12) 1283
Simultaneous determination of ofloxacin and ornidazole in pharmaceutical preparations by capillary zone electrophoresis
A simple, rapid and validated capillary electrophoretic method has been developed for the separation and determination of ofloxacin and ornidazole in pharmaceutical formulations with detection at 230 nm. Optimal conditions for the quantitative separations were investigated. Analysis times shorter than 4 min were obtained using a background electrolyte solution consisting of 25 mmol/l phosphoric acid adjusted with 1 M Tris buffer to pH 8.5, with hydrodynamic injection of 5 s and 20 kV separation voltage. The validation criteria for accuracy, precision, linearity and limits of detection and quantitation were examined and discussed. An excellent linearity was obtained in concentration range 25-250 μg/ml. The detection limits for ofloxacin and ornidazole were 1.03 +/- 0.11 and 1.80 +/- 0.06 μg/ml, respectively. The proposed method has been applied for the analysis of ofloxacin and ornidazole both individually and in a combined dosage tablet formulation. The proposed validated method showed recoveries between 96.16 and 105.23% of the nominal contents

Semreen MH, Aboul-Enein HY*// *Natl Res Ctr, Pharmaceut & Med Chem Dept, EG-12311 Cairo, Egypt
J Liq Chromatogr Relat Technol 2010 **33** (2) 191
Experimental design strategies in LC method development and in robustness testing for reversible cholinesterase inhibitor rivastigmine in pharmaceutical formulation
The quantitative determination of rivastigmine hydrogen tartrate, in bulk drugs and in pharmaceutical formulation has been achieved following development of an isocratic, reversed phase liquid chromatographic (RPLC) procedure. Chromatographic separation was accomplished on a Vertex Hypersil reversed phase C18 column (25 cm x 4.6 mm i.d., particle size 5 μm) employing acetonitrile and phosphate buffer. The protocol was validated by linearity (correlation coefficient = 0.9983), accuracy, robustness, and intermediate precision. An experimental design was utilised during validation to calculate procedure robustness and intermediate precision. Three factors were considered in the robustness test. These were percentage v/v of acetonitrile, flow rate, and temperature; an increase in flow rate resulted in a decrease of the concentration of the drug from the expected value, while the percentage of organic modifier and temperature had no effect on the response. For intermediate precision measurement, the considered parameters were analyst, equipment, and days. The RSD value (1.10%, n = 24) indicated a good precision

Silvestre CIC, Segundo MA*, Saraiva MLMFS, Lima JLFC// *Univ Porto, Fac Farm, Serv Quim Fis, ReQuimTe, Rua Anibal Cunha 164, PT-4099-030 Oporto, Portugal
Spectrosc Lett 2009 **42** (6-7) 341
Indirect sequential injection enzymatic determination of allopurinol in pharmaceuticals based on xanthine oxidase inhibition
The enzymatic determination of allopurinol in pharmaceutical formulations has been achieved following development of two automatic sequential injection analysis procedures. Both techniques relied upon the inhibition by allopurinol of the reaction catalysed by xanthine oxidase as assessed by spectrophotometric detection of the reaction product (uric acid) at 295 nm. The first procedure was established following a single readout after a fixed time period. The other was based on the decrease of the rate of formation of uric acid by monitoring the change in absorbance. The analytical performance of both procedures was validated and demonstrated low environmental impact tools for assessment of allopurinol in drugs

Souza MJE, Canedo NAP, Souza Filho PS, Bergold AM// Univ Fed Rio Grande do Sul, Fac Farm, Programa Posgrad Ciencias Farmaceut, Porto Alegre, RS, Brazil
J AOAC Int 2009 **92** (6) 1673
Development of an ultraviolet spectrophotometric method for the determination of ceftiofur sodium powder
The determination of ceftiofur sodium in the drug substance and sterile powder for injection has been achieved by development of a UV spectrophotometric procedure. Protocol validation, which produced acceptable data, included evaluation of the range, linearity, intra- and interday precision, accuracy, recovery, specificity, robustness, LOQ, and LOD. UV spectrophotometric analyses were performed at 292 nm. Good linearity was produced between 2.5 and 20.0 μg/ml. Prospective validation demonstrated that the method is linear (r = 0.9999) and precise, with RSD values of 0.3% for product A and 0.4% for product B. Intra- and interday precision values were < 2% for all samples. Comparison of UV spectrophotometry and LC by analysis of variance and Student's t-test showed no significant difference between techniques. The accuracy and precision obtained with the UV method compared well with the values obtained with the LC method. This correlation indicates that UV spectrophotometric analysis might be an inexpensive, reliable, and less time-consuming alternative to chromatographic analysis. Data illustrate the validity of the proposed technique as a simple and useful alternative for the determination of ceftiofur in routine QC analyses

Sultana N, Arayne MS, Shafi N, Siddiqui FA// Univ Karachi, Fac Pharm, Pharmaceut Sci Res Inst, PK-75270 Karachi, Pakistan
Chromatographia 2010 **71** (1-2) 71
Simultaneous RP-LC analysis of diltiazem and non-steroidal anti-inflammatory drugs in pharmaceutical formulations and human serum
The simultaneous analysis of diltiazem and non-steroidal anti-inflammatory drugs (NSAIDs) in the bulk drug, tablet dosage forms, and human serum has been achieved by the development of a simple, precise, accurate, selective, and sensitive reversed-phase LC-UV procedure. Chromatographic separation of the drugs was accomplished at ambient temperature on a C18 stationary phase with 80:20 (v/v) methanol-water, pH 3.1 +/- 0.02, as isocratic mobile phase. The mobile phase flow rate was initially 0.5 ml/min then increased to 1 ml/min. All the NSAIDs were clearly separated from each other and from diltiazem. Total run time was 10 min. The assay was successfully applied to pharmaceutical formulations and serum. There was no chromatographic interference from tablet excipients. The procedure was linear in the range 1.25-50 μg/ml both for diltiazem and the NSAIDs. The applicability of this HPLC procedure for quantitative analysis of the drugs was demonstrated by validation in accordance with International Conference on Harmonization (ICH) guidelines. Validation data, and those from statistical analysis illustrated that the procedure was reliable

Wenda C, Rajendran A*// *Nanyang Technol Univ, Sch Chem & Biomed Engn, 62 Nanyang Dr, SG-637459 Singapore, Rep Singapore
J Chromatogr A 2009 **1216** (50) 8750
Enantioseparation of flurbiprofen on amylose-derived chiral stationary phase by supercritical fluid chromatography
Enantiomers of flurbiprofen were separtated on an amylose-derived chiral stationary phase, Chiralpak AD-H, by supercritical fluid chromatography (SFC) under both linear and non-linear conditions. Pulse injections were employed using supercritical CO_2 modified with methanol as a mobile phase at a temperature of 30 °C. Under linear conditions, the isotherm was determined directly from the chromatogram. However, under overload conditions, the elution profiles were described by competitive Langmuir and bi-Langmuir isotherm. Isotherm parameters were calculated using the inverse method and the effects of operation variables, for example pressure and modifier composition, were determined. The value of selectivity is from 1.9 to 2.1 while the value of resolution was from 5.3 to 11.8. The number of theoretical plates was always greater than 5000 indicating high efficiency of SFC

Yeniceli D, Dogrukol-Ak D*// *Anadolu Univ, Fac Pharm, Dept Anal Chem, TR-26470 Eskisehir, Turkey
Chromatographia 2010 **71** (1-2) 79
Retention behaviour of bupropion hydrochloride in reversed phase ion pair LC and validated analysis of the drug in pharmaceuticals
A procedure has been developed for the analysis of bupropion hydrochloride (a basic drug of pKa 7.9) in pharmaceuticals based upon the results of an investigation into its chromatographic behaviour under reversed-phase ion-pairing conditions. Chromatographic separation of bupropion hydrochloride and carbamazepine (used as internal standard) was performed on a C8 column (150 mm x 4.6 mm i.d., 3.5-μm particle), with 40:10:50 (v/v) methanol-acetonitrile-phosphate buffer (20 mM, pH 3.0), containing 10 mM 1-heptane sulfonic acid sodium salt (1-HSA), as optimum mobile phase at a flow rate of 1.0 ml/min. UV detection was at 254 nm. The fully validated technique permits reproducible and selective analysis of bupropion hydrochloride in pharmaceuticals