Keyword Index

Presenting Author Index

Gelhaus, Stacy L	A77
Gergov, Merja	A49
Gerostamoulos, Jim	P25
Giroud, C	B3
Gordon, Ann M	C28
Goulle, J P	A22
Hackett, Jeffery	A23
Hamzah, Asimah	F23
Harden, Wat L	M4
Hayakawa, Hideyuki	P4
Hegstad, S	F21
Helander, A	A36
Hieda, Yoko	C14
Hirata, Yukari	A66
Hodda, A E	P65
Horak, Erica	A71
Huang, Wei	A84
Huestis, Marilyn A	M47
Humbert, L	C31
Itoh, Shinobu	A55
Jannetto, Paul J	A57
Jayashanker, G	C5
Johansen, Sys S	A78
Johnson, R D	P50
Jordan, S	A19
Juhascik, Matthew	B7
Jurado, C	M11
Kacinko, Sherri L	M51
Kala, Maria	B5
Kaneko, Rina	A59
Karch, Steven B	C34
Kardos, Keith	M48
Karlovsek, Majda Z	B6
Kaushik, Romina	A60
Kerrigan, Sarah	P57
Kiat, Wenceslo	C11
Kikura-Hanajiri, R	A61
Kim, Insook	M22
Kim, Jin Y	M41
Kinkennon, Amy	A31
Kintz, P	M13
Klys, Malgorzata	P9
Koski, A	P37
Kraemer, Thomas	B4
Kristoffersen, Lena	A45
Kronstrand, Robert	M14
Kucmanic, John J	A24
Kugelberg, F C	P3
Kuntz, David J	F2
Kupiec, Thomas	M24
Labat, L	F22
Laloup, Marleen	A50
Lambert, Willy	A79
Larson, Scott	F27
Lebrun, B	A12
Lechowicz, Wojciech	A34
Lemos, N P	P28
Leong, H S	M7
Lewis, Russell J	P42
Lim, Mi-ae	F15
Linden, Rafael	A62
Liu, Hsiu-Chuan	F16
Liu, Ray H	A63
Lood, Yvonne	A1
Lyle, Charles R	M21
Maralikova, Barbora	A13
Maresova, Vera	F25
Margalho, C	A14
Marinetti, Laureen J	P54
Martinez, Maria A	C24
Martins, Liliane	M10
Marti-Ortiz, A	A6
Maurer, Hans H	A38
Mayer, J	P26
Mazarr-Proo, Susan	P46
McGrath, Kelly K	P27
McIntyre, Iain M	P62
McKague, Kathleen	A40
McMullin, Matthew	F26
Meatherall, Robert	A64
Meenan, Gerard	A32
Merves, Michele L	M45
Mireault, Pascal	A52
Mobley, P L	F12
Monforte, Joseph R	F24
Moody, David E	F6
Mori, Tomohisa	B14
Moringa, Masatoshi	A51
Mozayani, Ashraf	P15
Muller, I B	A20
Mura, P	B1
Musshoff, F	M12
Mykkanen, Sirpa	A80
Namera, A	A7
Namiki, Mizuho	C17
Nebro, Wesenyalsh	M23
Nipper, H	P52
Noort, D	C23
Nunes-Daniel, F	P18
O'Hanlon, L	M1
Ojanpera, Ilkka	A69
Papa, Vincent M	F11

Parker, Dawn R	P56
Paterson, Sue	F9
Paul, Buddha D	A4
Peace, Michelle R	M44
Pehourcq, Fabienne	C6
Peters, Frank T	C21
Petrova, V	P41
Pichini, S	M46
Pirnay, S	P45
Poklis, Alphonse	C19
Poklis, Justin L	P34
Polettini, A	P29
Portman, M	B15
Pragst, Fritz	B2
Proenca, P	P14
Quintela, O	M25
Ragoucy-Sengler, Catherine	M26
Raikos, Nikolaos	A54
Rasanen, Ilpo	A21
Razatos, Gerasimos	A18
Rivadulla, Manuel	M27
Ropero-Miller, Jeri D	P19
Sahu, C R	C12
Saisho, K	A43
Samkova, H	P32
Sandberg, Michelle	P59
Sasaki, Tania A	A42
Schaffer, Michael	M8
Scheidweiler, Karl B	M28
Schur, B C	P60
Schwilke, Eugene W	M29
Scott, Karen S	M6
Shalan, M G	B16
Simonsen, K W	A44
Sklerov, Jason H	A25
Smith, AC	B9
Snyder, Ann	M50
Soper, John W	F10
Stankova, Marie	P1
Steenkamp, Paul A	P12
Steentoft, A	A8
Stimpfl, T	P16
Stripp, Richard	A65
Sutheimer, Craig A	F20
Swienton, Anjali R	A9
Tacker, Danyel H	M43
Takaichi, Kenichi	M30
Tasduq, S A	C18
Teixeira, H M	M31
Tsatsakis, Aristidis	M32
Tsujino, Yoshio	C15
Tyler, Mark	B17
Vallejo, Yli R	M33
Valouch, Tara J	P11
Van Bocxlaer, Jan F	A82
Verstraete, Alain	A3
Villain, M	M9
Vorce, Shawn P	A67
Vorisek, V	C1
Wada, Kentaro	C10
Wagner, Michael A	M34
Wan, Terence SM	A37
Wang, M	A53
Waumans, D	A26
Weinmann, Wolfgang	A68
Westenbrink, William	F13
Wicks, John	M35
Wille, Sarah	A81
Wilson, J M	C9
Wilson, Lisa	M36
Wolf, Carl E	C27
Wong, Raphael C	M37
Wong, Steve	P61
Woodall, Karen	P8
Wtsadik, Abraham T	M38
Wu, Anthony	F14
Yang, Wonkyung	M39
Yashiki, Mikio	C16
Yazzie, Janice	A27
Yong, C Y	M40
Young, Michael S	M42
Zaney, M E	A73
Zuba, Dariusz	A17
Zweigenbaum, Jerry	A28

Scientific Session Abstracts:

Analytical Methods

DETERMINATION OF PHENETHYLAMINE- AND PIPERAZINE-DERIVED DESIGNER DRUGS IN URINE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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New designer drugs have appeared on the illicit drug market during the last years. Many of these drugs are easily available on the Internet and there are several web sites that give a lot of information about these substances. Forensic laboratories are continuously in need of complementary addition of existing methods or developing new methods for these substances. Screening of urine samples for amphetamines in our laboratory is routinely done by enzyme immunoassay and those giving positiv results are confirmed by another analyze. The urine is analyzed by gas chromatography-mass spectrometry (GC-MS) in the selected ion monitoring mode (SIM) after liquid-liquid extration with isooctane followed by derivatization with trifluoroaceticanhydride. This method is quantitative for amphetamine, methamphetamine, MDMA, MDA and qualitative for MDEA, MBDB, phentermine, phenylpropanolamine, ephedrine, pseudoephedrine and phenmetrazine. If the result of this method is negative in spite of positive immunological testing or if the police or the pathologists have a request about other central stimulating designer drugs we use our method for phenetylamines and piperazines. It is a rapid and simple method because we use the sample extract from the former analysis and just reinject it on the GC/MS using the SIM. Verification of a positive result is done by full scan analysis and searching in our own reference library. The method was developed for identification of the phenetylamines *p*-methoxyamphetamine (PMA), *p*-methoxymethamphetamine (PMMA), 4-methylthioamphetamine (4-MTA), 3,4,5-trimethoxyamphetamine (TMA), 2,5-dimethoxy-4-methylamphetamine (DOM), 4-bromo-2,5-dimethoxyamphetamine (DOB), 4-chloro-2,5-dimethoxyamphetamine (DOC), 4-ethyl-2,5-dimethoxyamphetamine (DOET), 4-bromo-2,5-dimethoxyphenetylamine (2C-B), 4-iodo-2,5-dimethoxyphenetylamine (2C-I), 4-ethyl-2,5-dimethoxyphenetylamine (2C-E), 3,4,5-trimethoxyphenetylamine (mescaline) and the piperazines N-benzylpiperazine (A2), N-(3-trifluoromethylphenyl)piperazine (TFMPP), N-(4-methoxyphenyl)piperazine (MeOPP) and N-(3-chlorophenyl)piperazine (CPP). The selection of substances included in the method was dependent on the seizures done by the police and the availability of the substances. Seven of the substances were kindly received from the National Laboratory of Forensic Science, Linköping, Sweden. Immunoassay screening methodology is of limited value in detecting designer drugs in urine because of low cross reactivity for most of these substances. When a case, with clearly symptoms of being under the influence of central stimulant, needs to be investigated the described method is used even if the urine has been tested negative with immunological methods. In November 2003 one 17-year- and two 18-year-old males were found positive for 2C-E. They were caught by the police in the street showing symptoms that indicated ingestion of some narcotics. The urine samples were negative for screening but we analyzed further with the method for phenetylamines and piperazines and could by that identify 2C-E. The concentrations were 2,1 µg/mL, 0,8 µg/mL and 0,06 µg/mL respectively. Until today we have in 54 cases identified and verified seven of these substances in urine samples, 2C-E, MeOPP, A2, DOC, PMA, PMMA and 4-MTA.

Keywords: Designer drugs, Amphetamines, GC-MS

DETERMINATION OF AMPHETAMINE-RELATED DRUGS IN BIOFLUIDS BY GC/MS AFTER AQUEOUS-PHASE DERIVATIZATION WITH 2,2,2 TRICHLOROETHYL CHLOROFORMATE AND SOLID-PHASE MICROEXTRACTION

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The potential of 2,2,2-trichloroethyl chloroformate derivatization for the GC/MS analysis of a large range of amphetamine-related drugs (ARDs) and ephedrines in plasma, urine and hair samples has recently been demonstrated.
A new and simpler analytical approach has been developed for ARDs bioanalysis, based on aqueous-phase 2,2,2 trichloroethyl chloroformate derivatization, subsequent direct solid-phase microextraction (SPME) of derivatives, and their GC/MS detection.

Preliminary studies on amphetamine, MDMA, MBDB, TMA and DOB have been carried out to check feasibility of derivatization in aqueous conditions and to optimize reaction conditions. Comparative studies were undertaken to optimize SPME conditions: choice of SPME fiber, working pH, effects of salts, adsorption temperature, and adsorption and desorption times.

Results demonstrate that: ARDs can be derivatized in aqueous phase with 2,2,2 trichloroethyl chloroformate; carbamate derivatives can be extracted by direct immersion SPME; a simple and fast analytical procedure can be applied for sample preparation and GC/MS analysis (1.2 ml sample total volume, 5 µl derivatizing agent, reaction time 5 min, use of a PDMS 100 SPME fiber, buffered working conditions at pH 9, absence of salts, SPME adsorption temperature 30 °C and time 20 min).

The procedure has been successfully applied to the analysis of blank, spiked and true positive plasma, urine (0.5 ml) and hair (50 mg) samples.

Key words: Amphetamines, Chloroformates, SPME.

COMPARISON OF THE SENSITIVITY AND SPECIFICITY OF SIX IMMUNOASSAYS FOR THE DETECTION OF AMPHETAMINES

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Introduction: In drug of abuse screening, the ideal amphetamine immunoassay should detect amphetamine and the different illicit amphetamine analogues (e.g. MDMA, MDEA, MDA) without false positive results from anorectics, other stimulants or other drugs like ranitidine. We compared the sensitivity and specificity of 6 commercial urine amphetamine immunoassays for the analysis of the urine samples that were sent to our laboratory during a 2.5-month period.

Methods: Two hundred twenty five urine samples that had been sent to our laboratory for screening or confirmation of amphetamines were analyzed for amphetamines with the FPIA amphetamine/methamphetamine II assay (on Abbott ADx and AxSYM instruments), EMIT (Emit II Plus Monoclonal Amphetamine/Metamphetamine Assay and the new EMIT II Plus Amphetamines Assay) and KIMS (standard protocol and MDMA protocol, KIMS and KIMS X respectively). All assays were calibrated and used semi-quantitatively. All samples that screened positive by any amphetamine screening method and 15% of the negative samples were confirmed by LC-MS/MS. Briefly, 10 µL of urine was mixed with 90 µL of a mixture of deuterated internal standards and 20 µL was injected in the LC-MS/MS. The assay LOQ is less than 15 ng/mL of amphetamine, methamphetamine, MDMA, MDEA, MDA, 4-MTA and PMA. A sample was considered positive for amphetamines if any of these substances was present at > 200 ng/mL.

Results and discussion: Ninety-one (40%) of the samples were positive by LC-MS/MS. The number of positive samples, lowest, median and highest concentration (in ng/mL) are 74, 71, 2560 and 155000 for amphetamine, 1, 33, 33 and 33 for methamphetamine, 27, 46, 5975 and 108000 for MDMA, 23, 15, 516, 12400 for MDA and 4, 27, 1530 and 24800 for MDMA. MBDB, 4-MTA and PMA were not found.

Some characteristics of the different assays are given in the table:

	ADx	AxSYM	EMIT	EMIT N	KIMS	KIMS X
Area under the ROC curve	0.999	0.988	0.977	0.984	0.975	0.972
95% confidence interval	0.982-1.000	0.963-0.998	0.948-0.993	0.958-0.996	0.944-0.991	0.941-0.990
Optimal cut-off (ng/mL)	350	677	565	271	404	493
Sensitivity at the cut-off (%)	98.9%	95.6%	96.6%	90.9%	94.4%	93.3%
Specificity at that cut-off (%)	98.5%	97.8%	90.2%	100%	88.5%	89.3%
# false negatives at that cut-off	1	4	3	8	5	6
# false positives at that cut-off	2	3	13	0	15	14
# false negatives at 500 ng/mL	2	4	3	19	11	7
# false positives at 500 ng/mL	2	15	18	0	11	14

Discussion and conclusion: The best results were seen with the Abbott ADx assay that is not available anymore in Europe. If the cut-off is increased to 677 ng/mL, the AxSYM gives a low number of false positives and negatives. The new EMIT assay has excellent specificity, but misses more true positive samples: 2 samples containing amphetamine (225 and 253 ng/mL), 1 sample containing MDA (231 ng/mL), 4 samples containing MDMA (319-2760 ng/mL and MDA (113-516 ng/mL) and one sample containing amphetamine and MDMA). For the older EMIT assays and both KIMS methods, there was more overlap between negative and positive samples, resulting in a high number of false positives. The optimal cut-offs, calculated by analysis of the receiver operating characteristic curves, varied between 271 and 677 ng/mL. Use of 500 ng/mL cut-off doesn't change much for the ADx and KIMS X assays, increases the number of false positives for AxSYM and EMIT, and increases the number of false negatives for the new EMIT method and the KIMS method.

Keywords: Amphetamine, MDMA, Immunoassay

(R)-(-)-α-METHOXY-α-(TRIFLUOROMETHY)PHENYLACETYL CHLORIDE (MTPA) AS AN USEFUL CHIRAL REAGENT FOR ENANTIOMERIC SEPARATION AND QUANTITATION OF AMPHETAMINE, METHAMPHETAMINE, MDA, MDMA, AND MDEA IN URINE SPECIMENS

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In drug testing, the presence of methamphetamine in urine is generally confirmed by a GC-MS method. Prior to confirmation, the basic drug is derivatized to a suitable perfluoroalkylamide for better chromatographic separation. Once methamphetamine is detected, a second GC-MS test is necessary to distinguish positive results from medical use of Vick's inhaler or selegiline (Deprenyl). R-(-)-Methamphetamine is the urinary product from use of these medications. The second GC-MS test is to confirm the illicit use of (S)-(+)-methamphetamine. In the procedure, the two methamphetamine isomers are changed to the chromatographically separable diastereomers by a chiral derivatizing agent, (S)-(-)-trifluoroacetylprolyl chloride (TPC). But the method has inherent limitations. Racemization of the reagent produces mixed diastereomers even from pure (S)-(+)-methamphetamine. Instead of using TPC, we utilized (R)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA) to prepare the diastereomers of methamphetamine amide. The reagent showed no recemization because it contained no α-proton next to the carbonyl function. The method was extended to resolve GC peaks of (R)-(-)- and (S)-(+)-isomers of amphetamine, *3,4*-methylenedioxyamphetamine (MDA), *N*-methyl-MDA (MDMA) and *N*-ethyl-MDA (MDEA). Three ions from the drug and two ions from the deuterated internal standard were monitored to characterize and quantitate the drugs. For MDEA, only one ion was used. The compounds in urine after acid-base separation and solvent extraction were derivatized by (R)-(-)-MTPA and tested by the GC-MS. The quantitation was linear over 25 to 5,000 ng/mL for MDEA and 25 to 10,000 ng/mL for all other drugs. Correlation coefficients were >0.996. Precision calculated as the coefficient of variation at the calibrator concentration of 500 ng/mL was within ±11% for all drugs. The method was applied to test forty-three urine specimens. In 95% of the methamphetamine-positive specimens, only the (S)-(+)-isomer was detected. In all MDMA-positive specimens, the concentrations of (R)-(-)-isomer were greater than that of the (S)-(+)-isomer indicating longer retention of (R)-(-)-MDMA in the human body. In all specimens the total enantiomer concentrations (R + S) detected by the MTPA method compared well with that of a non-chiral method that used 4-carboethoxyhexafluorobutyryl chloride as derivatizing agent ($t \leq 0.650$, t_{crit} 2.069, N=24, 95% confidence). But the MTPA method has some advantage. It alone can replace the two GC-MS methods needed to confirm the presence of (S)-(+)-isomers of amphetamine and methamphetamine.

Keywords: Amphetamine/Methamphetamine, MDA/MDMA, Enantiomer separation

SURVEY ON FORENSIC TOXICOLOGICAL ANALYTICAL LABORATORIES IN BRAZIL

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Forensic Toxicology in Brazil is carried out, principally, by public laboratories, except for Doping and Workplace Drug Testing that are done in private laboratories. Compared to other countries, there are neither established guidelines to orient these analyses nor a system of certification to orient Toxicological Analyses, in general, and specifically for forensic purposes.

This paper had as objectives the following: to identify the laboratories that work with or have the potential to carry out tests pertaining to Forensic Toxicology and the respective assays, as required by the current public health legislation, as well as the methodologies used and the Quality Systems followed by this labs; to evaluate, beforehand, the technical capacity of these laboratories and what they must do in order to be qualified by the Brazilian Network of Laboratories in Public Health – REBLAS/ANVISA (the Brazilian Agency for Health and Sanitary Safety Vigilance and Management).

A survey was prepared of several assays carried out by the labs and were divided in three major groups: Group I – Analysis of Controlled Substances and Abusive Drugs in materials seized by the Police (*in natura*), with 120 assays; Group II – Presumptive Tests and Confirmation of Controlled Substances and Abusive Drugs in Biological Matrices *in vivo,* with 1000 assays; Group III – Presumptive Tests and Confirmation of Controlled Substances and Abusive Drugs in Biological Matrices *post mortem*, with 906 assays. Also included were a "Survey on Quality Systems" and a "Registration Card".

The surveys were prepared with the aim of dividing substances up into categories and respective sub-categories, with the most representative substance tests being included. The presumptive and the confirmation tests were included for all groups. Fifteen laboratories, representing all Brazilian geographic regions, took part in the research. The regions of South and Southeast Brazil were the most represented, each one with six participating laboratories, although the regions of North, Northeast and Middle-East Brazil also participated, with only one laboratory, however, presenting analyses of Group I. Of the 15 laboratories taking part in the research, only 9 of them answered to the Group I survey; 3 answered to both Group I and Group II; 2 laboratories answered only to Group II and only 1 laboratory, located in Southeast Brazil, declared that they had completely carried out the Group III survey.

Most of the laboratories taking part (80%) carried out the Group I assays– Analysis of Controlled Substances and Abusive Drugs. Group II – Presumptive Tests and Confirmation of Controlled Substances and Abusive Drugs in Biological Matrices *in vivo* was carried out by 33% of the institutions and Group III – Presumptive Test and Confirmation of Controlled Substances and Abusive Drugs in Biological Matrices *post mortem* was carried out by only one laboratory (6.6% of the laboratories studied). It was concluded that 26% of the laboratories taking part in the research still do not follow any of the quality parameters. The information from the Survey will serve as a basis for preparing policies, establishing and managing Forensic Toxicological Laboratories in Brazil.

Keywords: Brazil, Forensic Laboratories, Survey

APPLICABLE CRITERIA IN DETERMINING LIMITS OF DETECTION (LOD)

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The proposed HHS SAMHSA guidelines for the analysis of drugs of abuse will affect directly or indirectly a very large number of laboratories involved in workplace drug testing. The acceptance of the analysis of alternative matrices and the varied instrumentation used poses regulatory and forensic challenges. The criteria that a laboratory employs to attest as to the presence of drug are of the utmost importance. Chromatographic and mass spectral behavior of chemical species at the instrumental limit of detection is subject to variability as the method is being challenged at its limit of performance. A number of recent publications have suggested a "relaxation" of the chromatographic parameters and propose stressing more the mass spectral behavior of chemical species this could be problematic for forensic defensibility.

As the criteria used to determine the limit of detection will affect the limit of quantification and the lower limit of linearity of calibration curves and fundamentally the performance of the method it is of the utmost importance that a laboratory clearly and objectively define the criteria to apply. A comprehensive study using GC/MS was undertaken to revisit generally accepted chromatographic and mass spectral criteria at the limit of detection. Benzolyecgonine , 11-nor-Δ^9 - tetrahydrocannabinol-9-carboxylic acid (THC) and amphetamines were used to assess the following contributors on limits of detection determinations: matrix background , chromatographic profiles, mass spectral ion variability and statistical acceptance criteria. For example, we observed with benzolyecgonine chromatographic variations of the individual ion fragments and ion ratios that would be acceptable or not for the determination of LOD depending on the laboratory criteria.

Advances in analytical instrumentation and techniques are increasingly lowering the limits of detection. Acceptable criteria must comply with existing scientific principles and provide sound forensic evidence as to be useful in the detection of prohibited substances not only in workplace drug testing but also in laboratories where only the presence of the drug is required.

Keywords: LOD, Criteria, Forensic

QUANTITATIVE DETERMINATION OF AMPHETAMINES AND METHYLENEDIOXY-AMPHETAMINES IN URINE BY MONOLITHIC COLUMN AND HPLC-DAD

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Aims: A monolithic silica was developed for a new material of a micro or capillary scale HPLC. The material consists of continuous porous silica having a double-pore structure (through-pore and meso-pore). In this study, we developed a simple extraction of amphetamines and methylenedioxyamphetamines from a small volume of urine by using a capillary column packed with a C_{18}-bonded monolithic silica.

Methods: The mixture of sample urine (0.1ml), buffer and an internal standard was introduced into the monolithic capillary column using a gas tight syringe. Analytes were adsorbed on the column and endogenous interferences were washed with an acidified buffer. Analytes were then eluted with 20-micro-L of a mobile phase and direct injected into a HPLC.

Results: The recoveries of amphetamines and methylenedioxyamphetamines from urine were over 90%. The calibration curves showed linearity in the range of 100 – 50,000 ng/g in urine. The coefficients of variation of intraday and interday were below 10%.

Conclusion: These results demonstrated that the C_{18}-bonded column was useful for the extraction of amphetamines in urine. This column has a potential as a new tool for the extraction of chemicals in biological materials.

Keywords: Amphetamines, Methylenedioxyamphetamines, Monoliths

QUANTITATIVE DETERMINATION OF DIAZEPAM AND NORDIAZEPAM IN WHOLE BLOOD BY LC-MS/MS

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Aims: To present a full validated LC-MS/MS method for quantification of diazepam and nordiazepam in whole blood for routine use.

Methods: Blood samples were extracted with ethyl acetate at pH 9. After evaporation of the organic solvent, the residue was dissolved in solvent. Spiked blood samples (0.0025-2.5 mg/kg diazepam and nordiazepam) were used for the calibration curve. The analysis was performed on a Quatro micro MS/MS (Waters) coupled to an HPLC (Agilent). The separation column was a Zorbax SB-C8. The solvent consist of formic acid: ammonium acetate buffer pH 5.0: methanol (0.16:400:400). The masses: m/z 140 for nordiazepam and m/z 154 for diazepam were used for quantification.

Results: Detection limit was 0,001 mg/kg and the quantification limit was 0.002 mg/kg for both compounds. The calibration curves were linear in the measuring interval. The linearity was evaluated with polynomial regression. Within day precisions for blood controls spiked at 0.05 mg/kg, 0.5 mg/kg and 1.5 mg/kg (diazepam and nordiazepam) were <5%. Between day precisions for blood controls spiked at 0.05 mg/kg and 0.5 mg/kg were <10%. Recovery for spiked blood samples at 0.015, 0.25, 1.5 and 2.5 mg/kg was > 89%. The laboratory participates in an external quality control program. Ion suppression or other kind of interferences was not observed.

Conclusion: A validated method has been described. The method is accurate, precise, robust and useful for quantification of diazepam and nordiazepam in whole blood.

Keywords: Benzodiazepines, LCMS/MS, Validation

RESOURCE GUIDE FOR USERS OF SCIENCE AND TECHNOLOGY AND THE NATIONAL CLEARINGHOUSE FOR SCIENCE, TECHNOLOGY AND THE LAW

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New challenges for expert witnesses and the legal community have arisen due to recent developments in science and technology. New technologies and methodologies, as well as fields long considered established, such as latent print identification and tool marks are facing increased scrutiny. Given this explosion of scientific evidence litigation, scientists, law enforcement, laboratory personnel, judges and lawyers are overwhelmed by the amount of information required to educate them to meet these legal challenges. Until now, judges, lawyers, scientists and law enforcement personnel did not have one source that allowed them to navigate all the existing case law, journals, reports, proceedings and other resources necessary to conduct effective investigations and litigation. The *Resource Guide for Users of Science and Technology* was created to fill an information need specifically relating to legal issues implicated by the use of new technology in criminal and civil justice. Supported by a joint cooperative agreement between the NFSTC and NIJ (#2000-RC-CX-K001), the project developed a comprehensive searchable database from a variety of sources covering a wide range of topics. The database provides information on topics such as bloodstain pattern analysis, body scans/retinal scans, digital image enhancement, entomology, expert witness malpractice, fingerprints, questioned documents, smart cards, toxicology, trace evidence and tool marks. The Resource Guide covers existing court rulings, pending court cases, scientific and legal articles from applicable sources, relevant information from books, current and pending legislation, conference proceedings, university and continuing education courses, and pronouncements from professional organizations. The NFSTC/NIJ project produced a searchable CD. The information contained in the Resource Guide will be included in and expanded upon in the online resource being developed by the National Clearinghouse on Science, Technology and the Law at Stetson University College of Law. The Clearinghouse is supported by a grant from the National Institute of Justice (#2003-IJ-CX-K024). In addition to the development of the online resource, the Clearinghouse Program is building partnerships with law schools, professional associations and federal agencies, sponsoring a forensic science/science and technology seminar series, convening Community Acceptance Panels at the request of NIJ, developing training modules on a variety of forensic disciplines with an emphasis on distance education, and building a reference collection of law, science and technology literature available through interlibrary loan to other institutions.

Keywords: Science and law, Information, Toxicology

IDENTIFICATION OF BENZODIAZEPINES USING HPLC-ESI-MS-MS

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A confirmatory method for twenty-three commonly detected benzodiazepines in postmortem biological matrices has been developed using solid phase extraction (SPE) coupled with high performance liquid chromatography positive ion electrospray ionization tandem mass spectrometry (HPLC-ESI-MS-MS). Isolation and purification of the drugs was performed using mixed-mode SPE columns installed on a Zymark RapidTrace™ system. After addition of internal standard, samples were diluted with sodium phosphate buffer and extracted using UCT Clean Screen® SPE columns. The SPE columns were sequentially rinsed with 2% ammonium hydroxide (NH_4OH) in ethyl acetate, methanol, de-ionized water, and phosphate buffer. Samples were loaded onto the column at a flow rate of 2.0mL/min. The cartridges were then washed with de-ionized water, followed by a wash with 20% acetonitrile in de-ionized water, and dried under vacuum prior to elution with 2% NH_4OH in ethyl acetate mixture. Residues were reconstituted with the HPLC mobile phase and injected onto a Restek reversed phase C8 HPLC column. The analytes were eluted at a flow rate of 375µl/min with a solvent mixture composed of methanol:water containing 0.1% formic acid, and detected using an Advantage LCQ™ ion trap mass spectrometer. Positive ion ESI-MS resulted in ion spectra for each of the benzodiazepines at their expected retention time on the LC column. Unique MS-MS spectra for each of the benzodiazepines were obtained and matched the direct infused data acquired with neat standards (See Table I). The limit of detection for each benzodiazepine was estimated from extracted samples with decreasing concentrations. This method provides a rapid, sensitive approach to isolate, purify and confirm a broad spectrum of benzodiazepines.

Table 1 Benzodiazepine Spectral Data

Benzodiazepine	Molecular Ion $[M+H]^+$ (amu)	Product Ion (amu)	Benzodiazepine	Parent Ion $[M+H]^+$ (amu)	Product Ion (amu)
7-aminoclonazepam	286	250, 222	Estazolam	295	267, 192
7-aminoflunitrazepam	284	264, 256	Flumazenil	304	276, 290
7-aminonitrazepam	252	121, 114	Flurazepam	388	315, 288
Alphahydroxymidazolam	342	203, 324	Lorazepam	321	303, 275
Alphahydroxytriazolam	259	331, 279	Midazolam	326	291, 244
Alphydroxyalprazolam	325	297, 279	Nitrazepam	282	236, 254
Alprazolam	309	281, 274	Nordiazepam	271	243, 208
Bromazepam	316	288, 261	Norflunitrazepam	300	254, 243
Chlordiazepoxide	300	283, 241	Oxazepam	287	269, 259
Clonazepam	316	270, 251	Temazepam	301	283, 255
Desalkylfurazepam	289	261, 226	Triazolam	343	226, 308
Diazepam	285	257, 228			

Keywords: Benzodiazepines, HPLC, ESI-MS-MS

DIRECT ANALYSIS OF MDMA AND METABOLITES IN SALIVA USING CAPILLARY ELECTROPHORESIS

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Ecstasy, or 3,4-methylenedioxymethamphetamine (MDMA), has gained popularity among young adults in the past few decades mainly for its euphoric and stimulating effects. Though this drug has a "street" reputation of being safe, case studies have shown its toxic effects. Drug testing in recent years has focused on analysis of alternative biological specimens because of the invasiveness and ease of adulteration of the more commonly used blood and urine. Testing of saliva as an alternative biological matrix has advantages over conventional matrices: saliva sampling is noninvasive and the presence of MDMA in saliva correlates with individual being under the pharmacological effects of the drug. MDMA is a basic drug with an approximate pK_a= 9.9. At two pH units below the pK_a, MDMA is fully charged and amenable for separation by capillary electrophoresis. Analysis of MDMA in saliva by CE also does not require derivatization or extraction; consequently minimal sample pretreatment is required.

Capillary electrophoresis separates analytes by electrophoretic mobility, and allows a simplified rapid method for drug analysis. On-capillary detection in CE is generally performed by a diode array detector (DAD). Sample stacking is used to increase the sensitivity of the CE-DAD system. This paper demonstrates methods for the direct analysis of MDMA in saliva employing both CE-DAD as well as capillary electrophoresis/diode array/quadrupole time-of-flight mass spectrometry (CE/DAD/Q-Tof-MS). The DAD and Q-Tof-MS provide extremely sensitive on-line detection for CE as well as rapid component identification by UV-Vis or mass spectra. Limits of detection and reproducibility for the detection of MDMA and its primary metabolites will be discussed.

Key words: MDMA, Saliva, Capillary electrophoresis

ANALYSIS OF XTC SEIZURES IN THE COUNTY OF TOURNAI (BELGIUM)

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Introduction: By the early 1980s, ecstasy (XTC) was used as a recreational drug. Its consumption became particularly prominent in the United Kingdom, Spain, the Netherlands, Belgium and Germany. Recently, the dance culture is growing almost everywhere in Europe. Its usage was even described in less-festive events like athletic meetings. This led us to collaborate with the "Service Judiciaire d'Arrondissement" of Tournai for more than two years. In Belgium the presence of many "megadancing" is on the French – Belgian border. Relationship between cross-border consumption and cross–border traffic is not clear. Typological data (weight, logo, diameter, thickness, color...) does not provide any information regarding synthetic procedures used by the laboratories nor it provides the location of the clandestine laboratories. In order to control the drug trafficking, it is important to know the synthetic procedures used by the laboratories and also the distribution networks. In this study we investigated the profiling of impurities in ecstasy and spatial analysis of seizures that could provide useful information to the legal authorities in controlling drug trafficking.

Materials and methods: We analysed 2052 XTC pills received from 857 seizures realised in the County of Tournai. Each pill was examined for its colour, diameter, thickness, logo, and weight. The pills were then analysed for chemical impurities. Five milligram of the pill were treated by 1 ml of dichloromethane, mixed for 15 seconds, and centrifuged. The supernatant were separated and evaporated to dryness under nitrogen. The sample was then dissolved in 250 µl of methanol and analysed by GC-MS. Results were encoded in a database and the profiling was analysed. Similarities between seizures were recorded in a larger Europol database. Analytical data were also introduced into a geographical information system in order to identify clusters of similar profiles.

Results: The typological analysis showed that sometime logos were not used on the pills. In fact, we observed four logo-use patterns: 1) period for less than a month, 2) for several months, 3) for a year or 4) with a cyclical appearance. The pattern depends not only on the consumer (bad pills identified very fast) but also on the trafficker (capacity of production, material available). These observations provide information about the behaviour of dealers, traffickers, and tablet makers. The chemical impurities indicated primarily two synthetic routes used by the clandestine laboratories: one with reductive amination and the other with Leukart's method. The information allowed us to identify pills from different geographic locations and helped us to monitor and control drug trafficking.

Conclusion: Spatial, typological and chemical results enabled us to build a database. The information is helpful in identifying clandestine laboratories. It also provides information on the synthetic route used and the technical means employed to make the pills. This database permits to unveil links between different legal cases.

Keywords: XTC, Profiling MDMA

SCREENING FOR ACE INHIBITORS AND AT-II-ANTAGONISTS IN URINE BY LC-MS/MS

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For the detection of Angiotensin-Converting Enzyme inhibitors (ACE inhibitors: enalapril, lisinopril, perindopril, captopril, ramipril, cilazapril as prodrug and active drug) and Angiotensin-II-Receptor Antagonists (candesartan, eprosartan, irbesartan, losartan, telmisartan, valsartan) an LC-MS/MS target screening method has been developed to be used in clinical and forensic toxicological analysis. These drugs are widely used for lowering blood pressure besides betablockers, diuretics and calcium channel blockers - which have been the aim of our previous work [1] - and their intake after prescription or even the non-compliance of patients, not taking the drugs, could have severe effects on traffic safety. Furthermore, overdosage of these drugs can cause severe lowering of the blood pressure.

For method development by LC-MS/MS ESI and APCI mass spectra of the compounds have been compared. ESI was found to be more suitable in terms of sensitivity for the ACE inhibitors, whereas the AT-II-Receptor Antagonists were detectable by both ionization modes.

Linearity for quantitation was found for captopril, cilazapril, enalapril, lisinopril, perindopril, ramipril and ramiprilat in the range of 10 to 500 ng/mL with ESI after extraction from urine. LOD was below 10 ng/ml in urine. In patients' urine samples the ACE inhibitors were detectable in their active form (carboxylic form) by electrospray ionization after intake of therapeutic doses. However, the active form was not available as reference substance for all compounds and had to be generated by hydrolysis of the esters. The internal standard still remains a problem for quantitative analysis, since deuterated compounds are not available. For patients' urine and serum samples Benazepril was used as internal standard for quantitation, since it was not prescribed for these patients.

Method development for the sartanes included liquid-liquid extraction (LLE). LC-ESI-MRM was found to be suitable for target screening for these drugs after spiking to urine; irbesartan was detected in a forensic case.
Results of the method development and applications to patients' urine and also some serum samples are presented.

[1] Mueller CA, Baranda AB, Weinmann W. Screening for 1,4-dihydropyridine calcium channel blockers in plasma and serum by solid-phase extraction and LC-MS-MS. J Mass Spectrom (in Press, 2004).

Keywords: ACE inhibitors, AT II antagonists, LC-MS/MS

DETERMINATION OF FUROSEMIDE IN WHOLE BLOOD USING SPE AND GC/EI-MS

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Introduction: Furosemide (4-chloro-N-furfuryl-5-sulfamoylanthranilic acid) is an anthranilic acid derivative with strong diuretic potential. It exerts its action on the luminal side of the thick ascending limb of the Henle's loop by inhibiting chloride transport or sodium chloride co-transport. This loop diuretic is employed for the treatment of renal diseases, congestive heart failure and hypertension. Although furosemide is included on the list of prohibited doping substances, indicated by the World Anti-Doping Agency (WADA), it is widely used in sports, mainly in competitions classified by weight or to avoid detection of other drugs.
The aim of this work was the development and validation of a methodology for application in forensic toxicology. A simple, rapid and validated gas chromatography-electron ionization-mass spectrometry (GC/EI-MS) method is described to evaluate the use of whole blood in the quantitation of furosemide. A solid-phase extraction (SPE) technique was used to extract this compound from blood samples.

Materials and Methods: Stock solutions of furosemide and ketoprofen (internal standard) were prepared in methanol, protected from light and stored in the freezer at 4°C until use. *Extraction* - To 1 ml of whole blood were successively added 50 µl of internal standard solution (10 µg/ml) followed by furosemide at different concentrations (0.10, 0.25, 0.50, 1.00, 2.50 and 5.00 µg/ml). The mixture was vortex-mixed, sonicated for 15 min and centrifuged for 15 min at 3000 rpm. The sample was extracted using BondElut®-LRC Certify 300 mg SPE columns and the organic solvent evaporated to dryness in a vacuum rotary evaporator. *Selective derivatization* - The dry residue was dissolved with 25 µl of tetramethylammonium hydroxide 0.2 M (TMAH) and injected directly into the chromatographic system. *Chromatographic conditions* - Initial oven temperature was 160°C for 1 min, increased by 20°C/min to 270°C and held for 8.50 min. The temperatures of the injector and detector were set at 250°C and 280°C, respectively. The mass spectrometer was operated at 70 eV in the electron ionization (EI) mode using selected ion monitoring (SIM). The ions were monitored at *m/z* 81, 372 and 96 for furosemide and 209, 205 and 268 for ketoprofen.

Results: Calibration curves were measured daily, based in one-day assay protocol between 0.10 and 5.00 µg/ml, and correlation coefficients were above 0.9910. Control samples at low, middle and high concentrations of furosemide were measured in the same day. The calculated LOD and LOQ were 10.05 and 45.44 ng/ml respectively. Intraday precision (coefficient of variation - CV) was inferior to 7.9 % for all concentration levels. For the interday precision, the calculated CV was inferior to 13.9 % for all control samples. The relative recovery calculated for the three levels of the control samples (0.30, 0.75 and 3.00 µg/ml) were respectively 104%, 89% and 91%.

Conclusion: A sensitive, specific and reliable procedure has been developed for the determination of furosemide in whole blood samples. The experimental work was performed so that all validation parameters are considered simultaneously in one day of assay. We may conclude that the validated methodology is suitable for the application in forensic toxicology routine analysis.

Keywords: Furosemide, Whole blood, Solid-phase extraction

ANALYSIS FOR LORAZEPAM IN BLOOD AND URINE BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY - POSITIVE CHEMICAL IONIZATION

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Lorazepam is a benzodiazepine drug that is used to treat anxiety and seizures. It is also prescribed as a pre operative sedative. Because lorazepam is a powerful CNS depressant and can enhance the effect of other CNS depressants, effective procedures for the analysis of lorazepam in biological samples are essential. This study reports on the analysis for lorazepam by gas chromatography - mass spectrometry (GC-MS) in biological samples. Liquid – liquid extraction was performed on 1 mL sample volumes using clean, separate 16 x 100 mm silanized culture tubes with PTFE lined screw caps. Prior to extraction, the urine samples were hydrolyzed by adding 1 mL of 5000 units / mL β-glucuronidase in 0.1 M sodium Acetate, pH 5.0. The urine samples were incubated 3 hours at 40° C in an incubation oven. Following incubation, internal standard (100 µL of 1 ng/µL lorazepam-d_4) was added to the urine and unhydrolyzed blood samples. A 1 mL volume of saturated sodium borate buffer (pH 9) and 5 mL of toluene: dichloromethane (70:30) was added to each urine and blood sample. After mixing 20 minutes on a reciprocal shaker, the tubes were centrifuged at 2000 rpm for 10 minutes. The organic layer was collected into clean, separate 13 x 100 mm culture tubes. The extracts were evaporated to dryness under a stream at air at 40° C using the Turboevap evaporator. The extracts were derivatized by adding 100 µL of N,O-bis(trimethylsilyl) trifluoroacetamide +1% trimethylchlorosilane) to each tube. The extract tubes were heated at 70° C for 20 minutes by using a dry block heater. After heating, the extracts were cooled to room temperature and transferred to clean, separate autosampler vials. The derivative reaction incorporated 2 trimethylsilyl (TMS) groups on the lorazepam molecule. The derivatized extracts were analyzed by GC-MS using an Agilent 5973 GC-MSD system. Chromatographic separation was achieved using an HP-1 MS 30 M x 0.25 mm, 0.33 µm film capillary column (Agilent) and UHP helium (1 mL/min) as the carrier gas. The column oven temperature program was 125° C, hold 0. 2 minutes, 20° C/ minute to 300°, hold 7.5 minutes. Splitless injection with injection port temperature at 250° C was used. The transfer line temperature was set at 300° C. Positive chemical ionization was utilized with ammonia as the reagent gas and an ion source temperature at 200° C. Selected ion monitoring was employed to analyze (m/z) 465 for lorazepam-diTMS and (m/z) 471 for lorazepam-d_4-diTMS. The lorazepam-diTMS mass spectrum had a base peak at (m/z) 465 and the lorazepam-d_4-diTMS mass spectrum had a base peak at (m/z) 469. Since lorazepam-diTMS had a significant (m/z) 469 ion fragment, the (m/z) 471 ion was used to monitor for the lorazepam-d_4-diTMS. Calibration standards were prepared with blank bovine blood and ranged from 2.5 ng/mL to 1000 ng/mL. In house controls were prepared with blank blood at 3.5 ng/mL, 100 ng/mL, 650 ng/mL and with blank human urine at 100 ng/mL. In the intra-assay accuracy evaluation, the lorazepam concentration was within 13% of target. In the intra-assay precision evaluation, the coefficients of variation were within 8%. This method was used to analyze blood and urine samples from a post mortem case.

Keywords: Lorazepam , GC-MS, Positive Chemical Ionization

MATRIX DEPENDENT O-DEMETHYLATION OF 3-METHOXY OPIOIDS DURING DERIVATIZATION

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Several in-vitro conversions of compounds to their metabolites during the extraction process have been observed by various investigators. Among these are the conversion of methamphetamine to amphetamine, methadone to EDDP, cocaine to benzoylecgonine, and ethanol to acetaldehyde. These authors have observed the O-demethylation of the 3-methoxy opioids, codeine, hydrocodone and oxycodone to their respective metabolites, morphine, hydromorphone and oxymorphone, during derivatization with N-methylbistrifluoroacetamide (MBTFA). The transformation was observed to be dependent on specimen matrix, incubation time and incubation temperature. It is important for the toxicologist to be aware of this phenomenon when developing analytical assays and interpreting results.

Key words: Opioids, Demethylation, MBTFA

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AS AN ALTERNATIVE METHOD FOR COMPARATIVE ANALYSIS OF SEIZED DRUG SAMPLES

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The comprehensive testing of seized drug samples performed in forensic laboratories allows identification and determination of the controlled psychoactive substances, identification of additives (e.g. caffeine or paracetamol) and diluents (e.g. palmitylic acid or glucose), as well as chemical impurities. The results of analysis deliver information about synthesis methods applied by illegal producers. It is also possible to establish if the seizure drug samples originate from the same production batch or the same illegal laboratory and even to link the dealer with drug users. Hitherto, mainly gas chromatographic methods (GC/FID, GC/MS) are used for this purpose.

The aim of the present study was to develop an analytical procedure that makes possible separation of the substances occurring in the seized *ecstasy* tablets by means of the high-performance liquid chromatography (HPLC). The studies have been carried out on the samples of 3,4-methylenedioxymethamphetamine (MDMA, *ecstasy*) prepared by different routes: reductive amination, Leuckart reaction and safrole bromonation. The reductive amination was performed using various reducing agents: sodium cyanoborohydride, aluminium amalgam and sodium borohydride. In order to estimate the repeatability, each synthesis route was repeated three times. HPLC analyses were carried out on LaChrom D-7000 System (Merck-Hitachi) liquid chromatograph.

The solid-phase extraction (SPE) was used as the sample preparation method. First, 200 mg of *ecstasy* sample was dissolved in mixture of 1.5 ml of the ammonium buffer (pH 8.5) and 0.5 ml of 30 mg/l diphenylamine solution (internal standard). Than the suspension was vigorously shaken for 25 min at 2000 rpm. The optimisation of SPE conditions was focused on selection of the appropriate column filling, which enables to bind the main compound and to thicken the impurities that are important in view of comparative analysis. Bakerbond spe™ Octadecyl (3 ml, 500 mg) turned out to be the most effective. SPE was performed as follows: 1.5 ml of sample was rinsed with 6.0 ml of water and 0.3 ml of methanol and the impurities were extracted with 0.8 ml of methanol. Than, 0.3 ml of collected fraction was diluted with the phosphoric acid solution up to pH of 6.

Chromolith Performance RP-18 e (100 – 4.6 mm) monolithic column was used in HPLC analysis. Taking into account the variety of physicochemical properties of the impurities, the separation was done in gradient conditions. In order to optimise the resolution, the simplex method was applied. Four factors were taken into account which describe gradient conditions. After eight steps of optimisation the following conditions were selected: 0 min – 100% of phase A (water + 0.1 ml/l phosphoric acid), 21.3 min – 59% of phase A + 41% of phase B (acetonitryle), 32.7 min – 100% of phase B, 34 min – 100% of phase A. The flow was 1.0 ml/min. Diode array detector (DAD) was used.

The applied conditions of SPE extraction and HPLC analysis allow good separation of the characteristic impurities occurring in *ecstasy* samples. Because most of the impurities give similar UV/VIS spectra, it is impossible to identify them using diode-array detector. It is highly probable that application of LC/MS system will solve this problem. The obtained HPLC chromatograms could be treated equivalently to FID chromatograms where the impurities pattern is a subject to further chemometric analysis. The preliminary results show that the worked-out method allows to distinguish the samples prepared by different synthesis route as well as it could be useful in comparative analysis of seized drug samples.

The study was supported by the grant of the State Committee for Scientific Research, Warsaw, Poland, number 0T 00C 01024.

Keywords: Comparative analysis, Ecstasy, HPLC

EVALUATION OF A PORTABLE EVIDENTIAL BREATH ALCOHOL ANALYZER

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The Scientific Laboratory Division of the Department of Health acts by mandate as the regulatory agency for the Implied Consent Program for the State of New Mexico. The Laboratory is responsible for all blood and breath alcohol testing activities for law enforcement statewide. The geographical size and the nature of the state, characterized by a highly rural population, demands portable breath alcohol testing equipment. Moreover, future expansion and success of the breath testing program has focused on instrument portability and data management as critical issues amongst law enforcement agencies and the courts. Thus, the Implied Consent Section of the SLD evaluated the performance of the Intoxilyzer® 8000, a portable instrument, against the Intoxilyzer® 5000, a stationary instrument which is currently approved for use.

Instrument performance was evaluated at various alcohol concentrations, ranging from 0.04 to 0.55 g/dL in blood or g/210L breath. Special attention was placed on instrument performance at the *per se* and aggravated DWI levels of 0.08 g/dL and 0.16 g/dL, respectively, due to their legal significance. Precision and accuracy were evaluated using in-house ethanol controls in a wet bath simulator. Coefficients of variation using the Intoxilyzer® 8000 ranged from 0.30 – 1.3% (n=102), while CV ranges for the Intoxilyzer® 5000 were 0.7 – 2.1% (n=102). Calibration stability was assessed in addition to the distribution of data at concentrations between 0.04 and 0.55 g/210L. Accuracy was 100 – 102% for the Intoxilyzer® 5000 and 99-101% using the Intoxilyzer® 8000. Linear regression analysis of more than 700 comparative measurements revealed an R^2 of 1.000 ($y = 1.005x - 0.001$), where the Intoxilyzer® 5000 and the Intoxilyzer® 8000 were plotted on the y and x-axis respectively. Instrument response to mouth alcohol and volatile interferences was also investigated. Potential interferences were evaluated alone or in combination with ethanol using a wet bath simulator at 34.0°C.

The effects of extreme temperature and altitude were also examined using wet bath simulators and dry gas calibrant. Accuracy and precision were evaluated at high and low temperatures. High altitude performance was evaluated at 10,600 feet above sea level at a local ski resort. In addition to the scientific study, field evaluations were also conducted by law enforcement personnel. Based upon the results of the study, the Intoxilyzer® 8000 was approved as an evidential breath alcohol analyzer in the State of New Mexico.

Keywords: Intoxilyzer 8000, Breath, Alcohol

PERFORMANCE OF ASSAYS FOR THERAPEUTIC DRUG MONITORING AND URINE DRUGS-OF-ABUSE SCREENING ON A CONSOLIDATED WORKSTATION

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Introduction: The study objective was the assessment of performance of nine Therapeutic Drug Monitoring (TDM) assays and nine Drugs-of-Abuse assays (DAT) when processed in combination with simulated routine workload on Modular Analytics P (Roche Diagnostics).

Methods: All TDM and DAT assays involve the kinetic interaction of microparticles in solution (KIMS) except one enzymatic method. The study design comprises imprecision in simulated routine runs. The experiment tests for potential systematic or random errors by comparing the imprecision of reference results (standard batch) with results from samples run in a pattern simulating routine sampling. The study was supported by a program for computer aided evaluation (CAEv).

Results: Within-run CVs of the reference runs ranged from 2 to 5 % for the majority of TDM (Acetaminophen, Amikacin, Carbamazepine, Digitoxin, Gentamicin, Lidocaine, Phenobarbital, Phenytoin) and DAT assays (Barbiturates, Cannabinoids, Cocaine Metabolite, Methadone, Opiates, Phencyclidine, Propoxyphene). CVs up to 7 % were obtained for Digoxin, Amphetamines, and Benzodiazepines. Routine simulation resulted in slightly increased CVs of maximal 2 %. This is in agreement with experience from previous routine simulation studies.

Conclusion: With these results we conclude that no relevant reagent interactions occur on Modular Analytics P when using it as a consolidated workstation for TDM, DAT, serum proteins and general chemistry assays.

Keywords: Therapeutic Drug Monitoring, Drugs-of-Abuse-Screening, Consolidated Workstation

VALIDATION OF A HPLC METHOD FOR QUANTITATION OF MDMA IN TABLETS AND REPRESENTATIVE DRUG SAMPLING

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A method for quantification of MDMA (ecstasy) in powder/tablets using HPLC-DAD will be described. The powder was extracted with HPLC solvent (KH_2PO_4-buffer, pH 3.2:Acetonitrile (9:1)) in 5-10 minutes, and analyzed using HPLC-DAD. The column used was a Chromspher B5 column at 30 °C and 0,4 ml/min solvent flow. The identity was confirmed using UV-detection with a UV-curve fit of 900 or more and peak purity of 990 or more. The specificity of the method has been examined for other phenyl amines, and showed that only PMMA co-elutes with MDMA. The robustness of the method was good, and minor changes in solvent composition and solvent flow was possible. The method uncertainty was found to be 10%. The limit of detection was 0.001 mg/ml and the limit of quantification was 0.003 mg/ml.

Representative sampling of the ecstasy tablets was applied *prior* to MDMA analysis. Visual inspection was done to establish if a seizure was heterogeneous or homogenous. Heterogeneous seizures were divided in populations of similar appearance and were treated separately in further analysis. The validated method of MDMA analysis and sampling of tablets will be presented in the poster.

Keywords: MDMA, HPLC-DAD, Sampling.

ALCOHOL DETERMINATION BY HEAD SPACE DUAL COLUMN CAPILLARY GAS CHROMATOGRAPHY

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A head space dual column capillary gas chromatographic method was developed for the simultaneous determination of ethanol, methanol, acetone, isopropyl alcohol, ethyl methyl ketone and n-propanol in blood and urine. The method consisted of two identical instruments and conditions, but using different internal standards, 1-propanol and 2-butanol.

The gas chromatographs were 6890 Series GCs equipped with two flame ionisation detectors and ChemStation software (Agilent Technologies, USA). The autosamplers were Combi Pal Head space samplers (CTC Analytics, Switzerland). The parallel capillary columns were a Rtx-BAC1, 30m x 0.53mm, 3 µm film and a Rtx-BAC2, 30m x 0.53mm, 2 µm film (Restek, USA). The columns were connected to single injector with a Graphpack Divider (Gerstel, Germany). The head space conditions were as follows: incubation temperature 60 °C, incubation time 5 min (agitation speed 500 rpm), syringe temperature 65 °C, injection volume 500 µl. The GC conditions were as follows: split ratio 1:20, column flow rate 10.7 ml/min (He), injection port temperature 200 °C, oven temperature 45 °C (4 min), detector temperature 250 °C, H_2 flow 40 ml/min, air flow 450 ml/min, make-up flow (N_2) 45 ml/min. The total analysis time was 5 min 50 s.

A baseline separation was obtained for all six compounds on both columns. Thirty-eight compounds were tested for interference. Methyl acetate co-eluted with 1-propanol on Rtx-BAC1 and with acetone on Rtx-BAC2. No other compound was found to co-elute with an analyte on both columns. However, ten compounds co-eluted with an analyte on one column. The reporting limit was set to 0.2 mg/g for ethanol and 0.1 mg/g for the other analytes. The method was accredited for the determination of ethanol in blood and urine according to the standard SFS-EN ISO/IEC 17025. The uncertainty of measurement was 4 % for both blood and urine samples.

The present method takes advantage of the facility of the head space sampling by gas-tight syringe over loop or pneumatic systems. Two dual-column instruments with different internal standards comprise a reliable but still compact set up for forensic alcohol determination.

Key words: Head space GC, Blood alcohol, Capillary column

METAL AND METALLOID MULTIELEMENTARY ICP-MS VALIDATION IN WHOLE BLOOD, PLASMA, URINE, AND HAIR. REFERENCE VALUES

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Four multi-elementary metal and metalloid quantification methods using inductively coupled plasma mass spectrometry (ICP-MS) have been developed and validated in human whole blood, plasma, urine and hair using a single preparation procedure for each sample.

The ICP-MS measurements were performed using a Thermo Elemental X7CCT series and PlasmaLab software without a dynamic reaction cell. Twenty-four to thirty-two elements are simultaneously quantified in biological matrices: Li, Be, B, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Ba, W, Pt, Hg, Tl, Pb, Bi, U. 0.4 ml whole blood, plasma and urine sample are acid diluted with purified water prepared extemporaneously, triton X100 and butanol. In and Rh are used as the internal standards. For the urine samples, the results were corrected after enzymatic creatinine determination. 25 mg hairs are acid mineralized after a decontamination procedure and diluted as previously described for the biological fluids. To be validated, each element has to force: linearity with a correlation coefficient higher than 0.99. The intra-assay and inter-assay imprecision measured as the CV should be below 5% and 10% respectively. Global performance was assessed by quality control program. Our laboratory is a registered participant of the Institut National de Santé Publique du Québec (Sainte-Foy, Canada) inter-laboratory comparison program for whole blood, urine, and beard hair of non-occupationally exposed individuals spiked with selected elements.

Multi-element metal and metalloid analysis has been validated for 24 elements in the whole blood, 27 elements in the plasma, 29 elements in the urine and 32 elements in the hair, from 0 to 25, 250 or 1000 ng/ml owing to the element. Quantification limits range from 0.002 ng/ml (U) to 4.4 ng/ml (B) for the whole blood, from 0.002 ng/ml (U) to 7.7 ng/ml (Al) for the plasma, from 0.001 ng/ml (U) to 2.2 ng/ml (Se) for the urine, and from 0.2 pg/mg (Tl) to 0.5 ng/mg (B) for the hair. Normal values have been determined in the whole blood, the plasma, the urine (n=100), and the hair (n=45) based on healthy volunteers, leading to approximately 10,000 analyses. Al results are presented and discussed. Some clinical toxicology and forensic toxicology applications are reported.

ICP-MS has made significant advances in the field of clinical biology, particularly important in toxicological analysis. This is due the use of extremely effective equipment that permits better clinical and forensic toxicological analysis of metals and metalloids status of each individual.

Keyswords: Inductively coupled plasma, Mass detector, Metalloids

THE EXTRACTION AND ANALYSIS OF FLUNITRAZEPAM FROM WHOLE BLOOD/URINE USING A NOVEL SOLID PHASE EXTRACTION PROCEDURE AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-PHOTODIODE ARRAY DETECTION

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Flunitrazepam, has been well recognized as a drug of concern within the forensic toxicology community for many years. Several methods have been published for the analysis and detection of the drug previously.[1-3] The method described in this poster takes a novel yet simplified approach to the extraction/ detection of flunitrazepam in biofluids. Solid phase extraction followed by high performance liquid chromatography is employed without the need for a mass spectrometry unit for detection of low levels of the drug. Data is presented to exhibit the efficiency of the method in terms of limit of detection and quantitation.

Blood and urine were spiked with both the drug (flunitrazepam) and an internal standard (Prazepam) over a range of concentrations (0-10ng mL^{-1}). The samples were buffered with a small volume of 0.1M acetic acid and applied to commercially available mixed mode (C_8/SCX) columns that had been previously conditioned. The columns were washed, dried and eluted twice, once using ethyl acetate (3 mL) and again using an elution solvent containing ethyl acetate/ acetonitrile/ ammonia (3 mL). Both portions of the eluates were collected and combined. Evaporation was performed at room temperature. The residue was dissolved in 100 µl of 0.1% aqueous trifluoroacetic acid.

LC Conditions:

Column:	C_{18}, 150 x 2.1mm, 3.5µm
Mobile phase:	0.1% Trifluoroacetic acid/ Acetonitrile (67: 33)
Flow Rate:	0.3 mL min^{-1}
Column Temperature:	40°C
Detection:	220, 250 nm
Injection Volume:	50µL

References:

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Keywords: Flunitrazepam, Solid-phase extraction, Blood and urine

OHIO DEPARTMENT OF HEALTH EVALUATION OF THE INTOXILYZER 8000

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Recently, a new Breath Alcohol testing instrument was evaluated by the Ohio Department of Health. The Intoxilyzer 8000 is a smaller shielded plastic body breath alcohol analyzer that utilizes a pulsed infra-red energy source as well as makes use of a primary filter in the nine micron range to quantify alcohol. The instrument is engineered with the latest generation of power converters. This technology significantly reduces the weight of the instrument so that it can easily be carried using the attached handle. Furthermore, the instrument can operate in a limited fashion with an optional battery pack or continuously with a 12 VDC source such as a cigarette lighter plug. No electrical inverter is necessary to operate the device in mobile situations.

We examined several properties of this unit. Specifically, we looked at the in-vitro performance of two like instruments. Nine solutions were prepared in eight liter carboys to contain a range of concentration of ethanol and or acetone while purified water was used to evaluate a negative sample. The prepared solutions were analyzed by the Bureau on a Hewlett Packard 6890/7693 gas chromatograph using a dual capillary column headspace method. Standards were prepared from SRM 1828a and reagent grade acetone. All simulators used throughout the study were certified by the manufacturer prior to the evaluation. Furthermore, simulators were dried and replenished with a new bottle of solution after every twenty tests. As a consequence of rapid acetone depletion, acetone solutions were replaced after every ten tests. Transfer hoses which were connected to the calibration inlet during the evaluation were purged with dry air after every five tests. This procedure minimized the build up of excess condensate that would accumulate over the course of testing.

Mean concentrations of ethanol reported and the % coefficient of variance did not deviate by more than 5% in both instruments. A regression analysis between the mean alcohol concentration of twenty tests and the target concentration indicated a correlation of greater than 0.999 for both instruments over the respective concentrations. Prepared solutions containing acetone at a clinically significant concentration with or without ethanol were detected as an interferent for both instruments. Our results correlated well with the NHTSA evaluation of the Intoxilyzer 8000 which examined ten replicates of five ethanol concentrations of 0.020, 0.040, 0.080, 0.160, and 0.300g/210L.

In conclusion, the results from the in-vitro test performed on the Intoxilyzer 8000 instrument demonstrate that it is accurate, precise, and linear at the concentrations of ethanol tested. Furthermore, the results demonstrate that the unit is able to detect the presence of acetone vapors or a combination of acetone and ethanol vapors at the respective concentration for each volatile.

Keywords: Intoxilyzer, Alcohol, Breath

A RAPID METHOD FOR MEASURING ANTI-DEPRESSANTS IN POSTMORTEM BLOOD USING DUAL COLUMN LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

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A dual column, liquid chromatography mass spectrometry method was developed for the analysis of eleven anti-depressants and metabolites in 200uL of postmortem blood. Sample preparation consisted of internal standard addition, protein precipitation, and microfiltration. Filtrates were injected onto an extraction column (Oasis® HLB, 20 x 2.1mm, d_p=25um) and washed with deionized water, 20% acetonitrile, at 3mL/min. The trapped analytes were backflushed through a 10-port switching valve onto an analytical column (Zorbax® SB-C18, 30 x 2.1mm, d_p=3.5um) using a gradient of acetonitrile (20-80%) and 0.1% formic acid at a flow rate of 0.5mL/min. The positive, pseudomolecular ions of imipramine, desipramine, amitriptyline, nortriptyline, fluoxetine, paroxetine, fluvoxamine, trimipramine, N-desmethyl-trimipramine, clomipramine, and N-desmethyl-clomipramine were monitored following electrospray ionization. Overall instrumental analysis time was 11 minutes per sample including extraction and detection. Sample carryover, assessed by area% of signals in blank injections, was < 1.4% by the use of both a needle wash phase and injector programming. Linear ranges varied within a 0.025-1mg/L range and the online extraction recovery was between 75-105%. Additional time savings could be gained by the direct injection of diluted plasma, however, due to the inconsistencies in sample collection and the potential for signal suppression, a protein precipitation step was adopted for this work.

Keywords: Anti-depressants, Dual-column, LC/MS

1-(4-METHOXYPHENYL)-1-PROPANAMINE AS CONTAMINANT IN 1-(4-METHOXY-PHENYL)-2-PROPANAMINE (PMA) PREPARED VIA THE LEUCKART REDUCTIVE AMINATION METHOD

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Introduction: 1-(4-Methoxyphenyl)-2-propanamine (4-methoxyamphetamine, PMA) is an amphetamine analogue (co-)responsible for a series of lethal drug accidents the last few years. It is often traded as MDMA (N-methyl-1-(3,4-methylenedioxyphenyl)-2-propanamine) and facilely prepared from anethole, the main component of (star) anise oil. From a forensic point of view, the profiling of synthesis impurities that can be attributed to a specific synthetic pathway is very important. The following presents 1-(4-methoxyphenyl)-1-propanamine as a new PMA impurity. It is formed *via* the Leuckart reductive amination of 1-(4-methoxyphenyl)-1-propanone, an impurity arising from the peracid oxidation of anethole.

Methods: Synthesis: The performic oxidation reaction of anethole, the pinacol-pinacolone rearrangement and Leuckart reductive amination were performed using standard reaction conditions. 1-(4-methoxyphenyl)-1-propanone was prepared quantitatively *via* a solventless Friedel-Crafts acylation reaction of anisole with lithium perchlorate as catalyst. *GC/MS analysis:* Agilent 6890 Plus GC coupled to Agilent 5973N mass selective detector (EI, 36 – 400 *amu*, 70 eV). Column: Varian VF5-MS FactorFour capillary (30 m x 0.250 mm x 0.25 µm). Injection and oven programming: 1 µL injection (split 1:10); 50°C (hold 1 min), 5°C/min to 270°C (hold 15 min). Carrier gas: He (1 mL/min).

Results: The peracid oxidation of anethole yields anethole glycol (and esters), which is converted to 1-(4-methoxyphenyl)-2-propanone by refluxing in a methanol/sulfuric acid mixture. Also, we have demonstrated that 1-(4-methoxyphenyl)-1-propanone is formed as a contaminant during the peracid oxidation of anethole. Both substances can serve as substrate in the Leuckart reductive amination reaction, where 1-(4-methoxyphenyl)-1-propanone will yield 1-(4-methoxyphenyl)-1-propanamine and 1-(4-methoxyphenyl)-2-propanone will yield 1-(4-methoxyphenyl)-2-propanamine. When examining confiscated PMA preparations, 1-(4-methoxyphenyl)-1-propanamine is found to be present next to PMA, hereby giving an indication for anethole's application as precursor.

Conclusion: 1-(4-Methoxyphenyl)-1-propanamine is a new impurity formed during the Leuckart reductive amination reaction of I-(4-methoxyphenyl)-1-propanone, a contaminant produced in the peracid oxidation reaction of anethole. Its formation is demonstrated by a simulated synthesis, and its applicability is proven by its presence in confiscated clandestine PMA samples. There are only few data available on the pharmacology and toxicity of this newly discovered impurity.

Keywords: Profiling, PMA, 1-(4-Methoxyphenyl)-1-propanamine

EFFECT OF SODIUM CHLORIDE ON HEADSPACE BLOOD ALCOHOL ANALYSIS BY GC-FID

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Sodium chloride is frequently utilized as an additive for headspace alcohol analysis. The addition of salt to the mixture in the headspace vial increases the partial pressure of volatile compounds including alcohol and the internal standard. This is advantageous because the concentration of alcohol in the vapor phase is a function of both the temperature and the concentration of alcohol in the liquid phase.

The effect of salt was investigated in a series of experiments in which sodium chloride was added to the internal standard solution containing *n*-propanol and *t*-butanol. Ethanol was quantitatively determined using an Agilent HP 6890 GC equipped with dual capillary columns and a flame ionization detector (FID).

In-house and external whole blood controls were used for comparison purposes. When no salt was added, CVs ranged from 1.3 to 4.0% for alcohol concentrations between 0.04 and 0.30 g/dL. By comparison, CVs using the salt solution ranged from 1.2 to 4.2%. Accuracy was 100-105% and 99-109%, when either salt or no salt was added, respectively. A comparison of quantitative values obtained by both methods showed that the results were not statistically different. A total of 80 antemortem and postmortem casework samples were included in the study. These ranged in concentration from 0 to 0.567 g/dL ethanol. Linear regression analysis showed an R^2 value of 0.996 and a mean difference of 0.001 g/dL between methods.

Keywords: Ethanol, Sodium chloride, GC-FID

HIGH THROUGHPUT SCREENING OF DRUGS OF ABUSE BY LC/MS

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Drugs of abuse are screened in blood and urine throughout the world by immunoassay. This technology has the advantages of high-throughput, inexpensive and generally applicable as a screening tool. However, there is a reasonable possibility for both false positive and false negative results. In addition, to be reliable the methodology must typically utilize a high concentration cutoff. In contrast, screening by a highly selective methodology as liquid chromatography/mass spectrometry (LC/MS) is less susceptible to false negatives and positives. Because of its sensitivity it has a much lower cutoff than immunoassay.

One argument for immunoassay lies in its ability to detect classes of compounds and therefore would detect designer drugs not listed as a sought compound. LC/MS screening for specific drugs of abuse would miss this. However, all immunoassay positives must be confirmed, as there is no indication of what the substance is. This step would, like LC/MS, miss the non-listed substance.

If an immunoassay were to produce a positive screen for amphetamines, there would be no indication of what the compound(s) was. However, LC/MS would provide the specific amphetamine and its concentration. A clinician reviewing these data in the screen can make assessment about dose and the possibility of confounding substances that may also be present. It is this additional data that makes the LC/MS screen both reasonable and desirable from a quality of results standpoint.

We will present how the Department of Clinical Pharmacology performs high-throughput drugs-of-abuse screening using LC/MS. The operation of the instrumentation and maintenance procedures will be described showing how large numbers of samples are analyzed side-by-side for both drugs-of-abuse and therapeutic drug monitoring. Additionally, the quality control processes needed to assure all results are accurate will be discussed. These processes encompass the sample handling, preparation, analysis, and reporting of almost one million determinations (equivalent immunoassays) per year run on 24 instruments operating seven days a week.

Keywords: Screening, Drugs-of-abuse, LC/MS

SIMULTANEOUS DETERMINATION OF COCAINE, COCAETHYLENE, AND THEIR POSSIBLE PENTAFLUOROPROPYLATED METABOLITES AND PYROLYSIS PRODUCTS BY GAS CHROMATOGRAPGY/MASS SPECTROMETRY

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Cocaine (COC) is abused by smoking, nasal insufflation, and intravenous injection, and it is also taken with ethanol. Therefore, it is important to determine concentrations of COC and its metabolites, ethanol analogs, and pyrolysis products for establishing the degree of toxicity, the possible ingestion of ethanol, and the possible route of administration. In this study, a sensitive and selective procedure is developed for the simultaneous analyses of COC, benzoylecgonine, norbenzoylecgonine, norcocaine, ecgonine, ecgonine methyl ester, *m*-hydroxybenzoylecgonine, anhydroecgonine methyl ester (AEME), anhydroecgonine (AECG), cocaethylene, norcocaethylene, and ecgonine ethyl ester in blood, urine, and muscle homogenate.

In the analysis, available deuterated analogs of these analytes were used as internal standards. Proteins from blood and muscle homogenate were precipitated with cold acetonitrile. After the removal of acetonitrile by evaporation, the supernatants and urine were extracted by solid-phase chromatography. The eluted analytes were converted to hydrochloride salts and derivatized with pentafluoropropionic anhydride and 2,2,3,3,3-pentafluoro-1-propanol. The derivatized products were analyzed on a gas chromatograph (GC)/mass spectrometer by selected ion monitoring.

This method was successfully applied in analyzing 13 case specimens from aviation accident pilot fatalities and/or motor vehicle operators. AEME concentrations found in the 13 specimens were consistent with those produced solely by the GC inlet pyrolysis of COC controls, suggesting that COC was not abused in these cases by smoking. Although AEME remains a potential marker for establishing the abuse of COC by smoking, AECG was not a useful marker because of its low recovery and GC inlet production from COC metabolites.

The developed procedure is unique because multiple analytes can be analyzed in urine, blood, and solid tissues by a single extraction with increased sensitivity through formation of hydrochloride salts and using a one-step derivatization.

Keywords: Cocaine, Metabolites, Pyrolysis Products, Gas Chromatography/Mass Spectrometry

RAPID ANALYSIS OF THC AND METABOLITES IN WHOLE BLOOD

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Extraction of whole blood samples for tetrahydrocannabinol (THC) and metabolites may be time-consuming, especially with liquid-liquid extraction methods. Recently, a novel solid-phase extraction (spe) method has been developed for extracting THC and metabolites from whole blood. This spe method uses a pipette tip that houses the solid-phase sorbent; the extraction method is referred to as disposable pipette extraction (DPX). For this application, a 5 mL DPX tip is utilized to provide the lower detection limits required for the analysis. For the DPX extraction, 1 mL blood was spiked with 10 ng of internal standard (d_3-THC and d_3-COOH-THC). The blood was vortexed with 2 mL acetonitrile then centrifuged. The supernatant was transferred to a clean tube and 4 mL DI water was added. Approximately 3.5 mL of the solution and 5 mL of air (for mixing) were drawn into the DPX tip with a syringe attachment. After waiting 45 seconds, the solution was dispensed to waste. The rest of the sample solution and 5 mL of air were drawn into the DPX tip, and after 45 seconds it was dispensed to waste. Subsequently, 300 uL hexane-ethyl acetate (50/50) and 5 mL of air was drawn into the DPX tip, and this solution was dispensed into a GC vial. Another elution with 300 uL of hexane-ethyl acetate was used and added to the GC vial. The solution was dried under nitrogen and heat (90°C). For the derivatization, 25 uL ethyl acetate was added to the dried vial, 25 uL BSTFA was added, and the solution was capped under nitrogen and vortex mixed. The solution was derivatized at 90°C for 20 min. The GC vial was decapped and the solution was transferred to a GC vial insert. The insert was returned to the same GC vial, capped, and placed on the GC autosampler for sample injection (2 uL splitless). The parameters for this analysis are crucial because of potential interfering peaks from sample matrix components. The GC/MS instrument (HP 6890 with HP 5973 MSD) was equipped with a 30 m GC column (RTX-5 ms, 0.25 mm ID, 0.2 um film). The oven temperature program started at 80°C for 1 min, then ramped at 40°C/min to 200 C, then ramped at 20°C/min to 240°C with the temperature held at 240°C for 5 min, then it was ramped at 20°C/min to 300°C and the temperature held for 8 min. The flow rate was set constant at 2 mL/min. It was found that this higher flow caused separation of the 371 ion of THC from a prominent interfering ion. For the MS conditions, selected ion monitoring was used and the ions m/z = 371, 374, 386, 389, 303, and 306 were scanned at 50 ms for THC and (int. std.), and m/z = 371, 374, 473, 476, 488, and 491 for COOH-THC (and int. std.). Using the above parameters, THC and COOH-THC at levels of 1 ng/mL were detected using 3 ions. A semi-automated extraction device is demonstrated to perform 12 extractions simultaneously in just minutes (not including protein precipitation and derivatization). Results of extractions from actual case samples are presented, and direct comparisons with standard liquid-liquid procedures are presented. It is found that DPX offers a much-improved method for the analysis of THC and metabolites from whole blood in terms of sensitivity, selectivity and speed of analysis.

Keywords: THC, GC/MS, Extraction

ANALYSIS OF TETRAHYDROCANNABINOL (THC) AND CARBOXY-TETRAHYDROCANNABINOL (CTHC) IN WHOLE BLOOD

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THC and CTHC were isolated from 1 mL of whole blood using SPEWare Cerex PolyChrom THC SPE cartridges (35 mg beds). Analytes were eluted, and then derivatized with PFPA. Samples were reconstituted in 50 ul of ethyl acetate, and 2 uL injected on an HP 5890 GC interfaced to a Finnigan TSQ 7000 MS/MS.

The MS/MS was used in the positive CI mode for THC, and negative CI mode for the CTHC, with methane as the reagent gas. The THC molecular ion at 461 m/z was selected in Q1, collided with Ar in Q2, and two daughter ions monitored in Q3. The CTHC did not generate a strong molecular ion (622 m/z), so a 602 m/z fragment was selected in Q1. This fragment was collided with Ar in Q2, and two daughter ions monitored in Q3. D3-THC and D3-CTHC were used as internal standards, and appropriate ions were monitored for these compounds. Analysis time was ~15 min per sample.

The method was shown to have a linear range of 1-50 ng/ml for THC and 5-50 ng/ml for CTHC. Precision and accuracy studies were conducted using known control materials manufactured in drug free blood with good accuracy and precision over the linear range. Interference studies indicated that neither ibuprofen (110 ug/ml), acetaminophen (110 ug/ml), salicylate (700 ug/ml), nor ethanol (63 ug/ml) affected the analysis. Forty authentic blood samples from previous casework were analyzed using this method. Twenty had screened positive, and had been confirmed by an outside laboratory. Nine had screened positive, were sent for confirmation, and were returned negative. Eleven had screened negative and had not been previously confirmed.

Keywords: Blood analysis, Carboxy-THC, Tandem mass spectrometry

OXYCODONE ABUSE CONCERN IN METHADONE MAINTENANCE PATIENTS

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Detection of oxycodone abuse is a significant problem for drug treatment facilities. A common misconception concerns the idea that oxycodone is a synthetic opiate and staff members believe that the urine screen for opiates detects oxycodone, also. For example, the amount of oxycodone that yields a positive opiates screen on the DRI Opiates Assay is greater than 16,000 ng/mL. The usual level in patients taking oxycodone is from 300 to 1,000 ng/mL.

Microgenics Corporation has developed a specific urine oxycodone homogeneous enzyme immunoassay. Evaluation of this DRI Oxycodone Assay was added to screening of methadone "intake" patients followed by GC/MS confirmation of tests positive for oxycodone. Microgenics has proposed either a 100 or 300 ng/mL cutoff level. The cutoff concentration used for this study was 100 ng/mL. The oxycodone immunoassay was tested on a Hitachi 717 Analyzer. 224 drug treatment "intake" patients' urine samples were screened with the specific oxycodone immunoassay. Using a 100 ng/mL cutoff level 45 urine samples screened positive for oxycodone yielding a positive rate of 20.1%. All presumptive positive samples were extracted through Biochemical Diagnostics GV-65 Detectabuse columns; derivatized by BSTFA with 1% TMCS; and analyzed by selective ion monitoring (SIM) on an Agilent Technologies 5973 GC/MSD system. The GC/MS confirmation cutoff was 25 ng/mL. 45 of the 45 samples confirmed positive for oxycodone demonstrating a 100% confirmation rate. The amount of oxycodone recovered in the urine samples by GC/MS ranged from 25 ng/mL to 37,116 ng/mL.

The DRI Oxycodone Assay identified 20.1% of the subjects as positive for the synthetic opiate. The specificity of the assay was 100% as determined by GC/MS analysis. Thus, in this population 20.1% of the Methadone Maintenance "intake" patients were using oxycodone. With the FDA approval of a generic sustained release oxycodone, abuse is likely to increase. The DRI Oxycodone EIA will be very useful in differentiating oxycodone from other opiate abuse.

Keywords: Oxycodone, Enzyme Immunoassay, GC/MS

EVALUATION OF BUPRENORPHINE CEDIA ASSAY USING SAMPLES FROM PATIENTS IN SUBSTITUTION TREATMENT

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Buprenorphine is used as an analgesic drug, and for detoxification and substitution therapy of opioid dependence. However, in combination with the increased medical use, buprenorphine also occurs on the black market as an illicit drug and fatalities due to poly-drug use has been reported. In addition, pausing from buprenorphine intake during opiate relapsing has been observed and can make compliance monitoring necessary. As a consequence there is a need for analytical service and toxicological monitoring of patients. However, there has been a lack of an immunoassay suitable for automated high-volume screening. The aim of this study was to evaluate the prototype buprenorphine CEDIA (Microgenics Inc.) by comparing it with existing ELISA (Diagnostix Ltd) and GC-MS methods. Urine samples were collected from patients in heroin substitution treatment with methadone, buprenorphine or dihydrocodeine. In total, 1552 samples were obtained from approximately 600 patients, consisting of 70% males and having an age range of 18-54 y. The CEDIA test was performed on Hitachi 911 and 912 (Roche Diagnostics, IN) instruments with a semiquantitative test protocol (five calibrators in the range 0-78 µg/L, sample volume 10 µL) supplied by Microgenics Inc. Samples with response >75 µg/L were diluted 10- or 100-fold with saline.

At the levels exceeding the 5 µg/L cutoff level, a variability lower than 10% was observed both within- and between-days. The correlation of CEDIA and ELISA was studied in 221 samples. There was a 96.8 % agreement in qualitative results between the methods. In three samples (2.7 % of all positives) CEDIA produced a false positive response as the GC-MS confirmation was clearly negative. In one sample the CEDIA result was false negative as the sample contained 26 µg/L of buprenorphine according to GC-MS. The agreement between the CEDIA and GC-MS methods in quantifying buprenorphine was studied in the range from 0 to 6000 µg/L. The samples were obtained from 72 patients receiving buprenorphine doses between 0.5 – 25 mg/day. The slope was 1.09 with an intercept of -12 on the CEDIA axis. The median ratio between CEDIA and GC-MS was 0.96 (n=298). By pooling data for all samples with a buprenorphine concentration >5 µg/L according to GC-MS or ELISA (n=400), the sensitivity for CEDIA of detecting positive samples at the cutoff level of 5 µg/L was calculated to be 99.5%. A total of 1011 samples from patients who were not receiving prescribed buprenorphine but received other heroin substitution treatments (mainly methadone) were used to estimate specificity. Thirty samples (3.0 %) were found to be positive by CEDIA at the cutoff level of 5 µg/L. One of these had detectable buprenorphine by GC-MS but at a low level (0.6 µg/L). Fourteen of the 30 samples were from patients prescribed dihydrocodeine and contained high amounts of this substance and metabolites. A further 20 samples from patients prescribed dihydrocodeine in daily doses of 14 –80 mg were therefore studied and all were positive in the CEDIA assay with responses ranging from 5 – 34 µg/L. The 15 false positive samples out of the 1011 (1.5%) all had a low response (<10) and all expect for one were highly positive in the CEDIA test for opiates, indicating that a cross-reactivity with codeine can be suspected.

In conclusion, the new CEDIA assay for buprenorphine is suitable for use in clinical routine testing at a cutoff limit at 5 µg/L.

Key words: Buprenorphine, Urine, CEDIA

RAPID DETERMINATION OF PSILOCIN IN WHOLE BLOOD BY LC-MS/APCI

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Psilocin and psilocibin are naturally occurring mushroom alkaloids, with their structure being similar to serotonin. Hallucinogenic properties of these compounds make them desirable for drug addicts especially for those who experiment with so-called "altered state of consciousness". However, there are many species of mushrooms similar to *psilocybe* but not containing psilocibin or psilocin. Ingestion of unknown mushrooms may lead to serious intoxication. Rapid method analysis by means of liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization (LC-MS/APCI) for detection and quantification of psilocin in whole blood has been proposed. Optimization of ionization and fragmentation parameters was performed using flow injection analysis (FIA). Total ion current (TIC) was recorded. Optimization was conducted for most abundant ions of m/z 205, 160, 115. Seven parameters were chosen for optimization: fragmentor voltage (60/120 V), capillary voltage (4000 V), vaporizer temperature (320°C), drying gas temperature (300°C), pressure (50 psig) and gas flow (7 L/min) and corona current (4.5 μA); (in parentheses optimal value is given). Liquid-liquid extraction using a mixture of n-propyl chloride:dichloromethylene (2:1, v/v) after blood alkalization (pH 11) was applied. Psilocin-D_{10} was used as the internal standard. Separation was carried out using chromatographic column, prepared according to customer requirement: LiChroCART (55x4 mm) filled with Superspher 60 RP-8e stationary phase. The stationary phase ensured appropriate retention: Psilocin – T_R=2.42 min; Psilocin-D_{10} – T_R=2.41 min. For quantification ramped SIM mode was applied. The pseudomolecular ions of m/z 205, 215 were recorded at fragmentor voltage of 60 V and fragmentation ions of m/z 160, 170, 115, 125 at 120V. The method was thoroughly validated. Intra- and inter-day precision (repeatability) for target concentration of 10 μg/L was 9% and accuracy measured for the concentration range of 4-40 μg/L, expressed as a mean recovery, was 102±15%. Results of three mushroom intoxication cases have been studied. Only in one tested blood sample did the concentration of psilocin exceed 5.2 μg/L, the level determined to be LOQ. The determined concentration was 8 μg/L of free psilocin and 12 μg/L of total psilocin. In case of two others person's concentration of free psilocin was above 1.7 μg/L (LOD) but lower than LOQ. Some important remarks concerning ion suppression caused by matrix, adsorption and solubility of psilocin have been shown to exert substantial influence on signal intensity.

Keywords: Psilocin, Blood, LC-MS/APCI

ETHYLGLUCRONIDE ANALYSIS IN URINE BY LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

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β-D-ethylglucuronide (EtG) is a metabolite of ethanol which is formed by the conjugation of ethanol with activated glucuronic acid in the presence of membrane-bound mitochrondrial UDP glucuronyl transferase. EtG is produced at approximately 0.02-0.06% of an ingested dose. EtG is stable, water-soluble and can be detected in urine and can be detected for up to three days following ethanol consumption. Urine ethanol levels are normally detected only for several hours, thus making EtG a unique metabolite for monitoring abstinence of alcoholics in alcohol treatment programs.

Urine samples for analysis are prepared by the direct addition of 100 µL of urine with 400 µL of internal standard (EtG-D5) in water into the liquid sample vial. No extraction is required. The HPLC used in the analysis is a Shimadzu SCL-10A with a Phenomenex Luna 3µm column with a mobile phase consisting of methanol, water and formic acid with a flow rate of 400µL/minute. The mass spectrometer is a Sciex API-3000 using a Turbo Ion Spray Source in the negative ion mode. Quadrupole 1 was set to pass the 221 and 226 ions for EtG and EtG-D5, respectively. Transition ions of 75 (quantitation) and 85 (qualifier) were monitored for each compound.

The assay was linear between 40 ng/mL to 10,000 ng/mL with a cutoff established at 100 ng/mL with a Limit of Detection and Limit of Quantitation of 40 ng/mL. Precision was determined with %CV of less than 1% between the concentrations of 40-150 ng/mL. The %CV between runs was determined to be less than 7%. Data from dosing studies, including incidental exposure, will be presented.

Keywords: Ethylglucuronide, Ethanol

MASS SPECTROMETRIC IDENTIFICATION OF ETHYL SULFATE IN HUMANS – A NEW ETHANOL METABOLITE AND A BIOMARKER OF ACUTE ALCOHOL INTAKE

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The traditional way to establish whether a person has recently consumed alcohol is by the analysis of breath, blood or urine samples for the presence of ethanol. However, because ethanol is rapidly metabolized and eliminated from the body, a method to reveal recent drinking even after ethanol has been cleared would have considerable importance in clinical and forensic toxicology. A very small fraction (<0.1%) of the ethanol consumed undergoes a phase II conjugation reaction catalyzed by UDP-glucuronosyltransferase to produce ethyl glucuronide (EtG), which is excreted in urine. This non-oxidative trace metabolite has attracted interest mainly as a specific biochemical marker for recent consumption of alcohol.

Animal studies have indicated that ethanol may also undergo sulfate conjugation to produce ethyl sulfate (EtS). In the present study on humans, a direct electrospray LC-MS method was used to confirm if EtS is formed following intake of alcohol and excreted in the urine. Urine samples were collected from healthy subjects at timed intervals after drinking a single ethanol dose. Urine samples were also selected at random from those sent to the laboratory for routine testing of recent alcohol consumption. Samples were stored at –20°C until analysis.

LC-MS analysis of EtS was performed in the negative ion mode, with selected-ion monitoring at m/z 125 for EtS (M_w 126.1 g/mol) and m/z 226 for EtG-d_5 (used as internal standard). The identity of EtS was confirmed by the correct S isotope ratio in authentic specimen, as compared to EtS reference material. The EtS concentration of unknown samples was determined from the peak area ratio of EtS to EtG-d_5, by reference to a calibration curve. The calibration curve was linear (r^2=0.99, $p<0.0001$) up to at least 800 µmol/L EtS and the detection limit was ~0.5 µmol/L.

After healthy subjects drank 0.5 g/kg ethanol, urinary ethanol peaked at 2 h and was no longer measurable at 8 h. EtS was detected in the sample collected at 1 h after intake and the peak value was obtained at 4 h and it was still measurable at 29 h. EtS showed a similar time course as EtG, with a mean EtG/EtS ratio of 1.5. Of the 54 clinical samples analyzed to date, all 31 with a detectable EtG were also positive for EtS (range 1.1-2095 µmol/L) and another 2 were only positive for EtS. These conjugated ethanol metabolites were highly correlated (r^2=0.84, $p<0.0001$) with somewhat higher concentrations noted for EtG. The remaining 21 clinical samples were negative for both EtS and EtG. Furthermore, no EtS was detected in urines collected from healthy individuals who had abstained from ethanol for several days prior to sampling according to self-report.

These results confirm that sulfate conjugation is a minor metabolic pathway for ethanol in humans and that EtS is a common constituent in the urine after alcohol intake. Potential applications of EtS as alcohol biomarker include detection of relapse or verification of abstinence during out-patient treatment of alcohol-dependent individuals and drunk drivers. In forensic toxicology, EtS may be used in workplace testing, such as after a serious accident (possible hangover effect), and to determine whether the ethanol identified originates from alcohol ingestion before death, or sampling, or was generated artifactually.

Keywords: Ethyl sulfate, Ethanol metabolism, Alcohol biomarker

HIGH THROUGHPUT SCREENING OF CORTICOSTEROIDS AND BASIC DRUGS IN HORSE URINE BY LC-MS-MS

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Introduction: Gas chromatography – mass spectrometry (GC-MS) has long been accepted as a powerful technique for the screening and confirmation of the presence of prohibited substances in biological samples from human and animal athletes. Over the past decade, liquid chromatography – mass spectrometry (LC-MS) has evolved into a mature technique and is gaining wide acceptance in many doping control laboratories. LC-MS or LC-MS^n is particularly suited for the analyses of polar, non-volatile and heat-labile drugs that cannot be adequately handled by GC-MS. In addition, tedious derivatization steps can often be omitted. This paper describes two high throughput LC-MS-MS methods for the screening of two important classes of drugs in equine sports, namely the corticosteroids and the basic drugs, at low ppb levels in horse urine. The method utilized a high efficiency reversed phase LC column (3 cm L x 2.1 mm ID with 2.5 μm particles) to provide fast turnaround times as well as achieving significant reduction in the consumption of expensive HPLC solvents. The performance of these two methods on real samples was demonstrated by analysing drug administration and positive postrace urine samples.

Method: Corticosteroids and basic drugs were extracted from enzyme-treated urine by solid-phase extraction using a Bond Elut Certify® cartridge and analysed by LC-MS-MS in multiple reaction monitoring (MRM) mode using a Thermo Finnigan triple quadrupole TSQ Quantum mass spectrometer. Separation of the corticosteroids and basic drugs was achieved using a short reversed-phase C18 column (3 cm L x 2.1 mm ID with 2.5 μm particles) on two different LC gradient solvent systems.

Results: Using the methods developed in this study, the detection of 23 corticosteroids and 42 basic drugs could be achieved within a 2.5-min and a 3.5-min LC-MS-MS run respectively. The overall turnaround time for the corticosteroid screen was 5 minutes and that for the basic drug screen was 8 minutes, inclusive of post-run and equilibration times. The results on the analysis of drug administration and positive postrace urine samples also demonstrated that both methods were effective in detecting corticosteroids and basic drugs in horse urine at low ppb levels.

Conclusion: Two high throughput LC-MS-MS methods with the use of a high efficiency LC column have been developed for the screening of corticosteroids and basic drugs at low ng/mL levels in equine urine. Validation data will also be presented. These methods can be used to control the abuse of these two classes of drugs in racehorses.

Keywords: Corticosteroids, Basic drugs, High throughput LC-MS-MS

SCREENING FOR, LIBRARY-ASSISTED IDENTIFICATION AND FULLY VALIDATED QUANTITATION OF TWENTY-TWO *beta*-BLOCKERS IN BLOOD PLASMA BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY WITH ATMOSPHERIC CHEMICAL IONIZATION

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Background: Overdose of beta-blockers may lead to life-threatening situations. Although there is no strong correlation between plasma concentration of beta-blockers and their pharmacological and toxic effects, suitable analytical procedures are necessary for toxicological screening, identification and quantification in clinical and forensic toxicology. Therefore, an assay was developed for such purposes in human blood plasma using LC-APCI-MS.

Methods: After mixed-mode (Confirm HCX) solid-phase extraction of 0.5 mL of plasma, the beta-blockers were separated on a Superspher 60 RP Select B column (125 x 2 mm I.D., guard column: 10 x 2 mm I.D.) using fast gradient elution (ammonium formate buffer/acetonitrile). The compounds were screened for and identified using an LC-APCI-MSD (SL version) in the scan mode with fragmentor voltages of 100 and 200 V by mass chromatography with selected ions followed by library search of the underlying full APCI mass spectra with our new LC-MS reference library. The drugs were quantified in the SIM mode at 100 or 200 V using calibration curves. The assay was fully validated according to internationally accepted recommendations.

Results: The assay allowed screening for, library-assisted identification (both in scan mode) and quantification (selected-ion mode) of the beta-blockers acebutolol, diacetolol, alprenolol, atenolol, betaxolol, bisoprolol, bupranolol, carazolol, carteolol, carvedilol, celiprolol, esmolol, labetalol, metoprolol, nadolol, nebivolol, oxprenolol, penbutolol, propranolol, sotalol, talinolol and timolol in blood plasma. The assay was found to be selective for all tested compounds. No interfering peaks were observed in the extracts of ten different blank plasma samples. Interferences with common drugs typically taken in combination were tested and could be excluded due to different retention time and/or mass spectra. The assay was linear from sub-therapeutic to overdose concentrations of all compounds. A weighted ($1/x^2$) least squares model was used for calculation of calibration curves. Low and high level recoveries ranged from 46.9% to 94.8% for all studied analytes. LODs were determined in the full scan mode and they were all lower or equal to the corresponding LOQs in the SIM mode. The LOQs corresponded to the lowest calibrator concentrations with a signal-to-noise ratio of at least 10. Within-day, between-day and total precision (combination of within- and between-day effects) lay within the required limits of ≤15% RSD (≤20% RSD at LOQ). Accuracy data also all lay within the acceptance interval of ±15% (±20% at the LOQ) of the nominal values. In processed samples, the analytes were stable for a period of more than 24 hours at room temperature. No instability of analytes in spiked samples was observed over three freeze/thaw cycles or during storage at –20 °C for a one month period. The procedure has proven to be applicable in the analysis of authentic plasma samples during routine work. The presented assay is the first fully validated procedure for the simultaneous determination of a large number of beta-blockers in plasma. In emergency toxicology, it should be applicable to confine to one point calibration because the y-intercepts of the calibration curves were either not significant ($p < 0.05$) or small compared to the response at high therapeutic concentrations.

Conclusions: The LC-MS assay has proven to be appropriate for screening, identification and quantification of beta-blockers in plasma after intake of therapeutic as well as of toxic dosages. It was successfully applied to authentic plasma samples allowing confirmation of diagnosis of overdose situations as well as monitoring of patients' compliance.

Keywords: liquid chromatography-mass spectrometry; beta-blockers; plasma

RAPID DETERMINATION OF CHLORAMPHENICOL AND ITS GLUCURONIDE IN FOOD PRODUCTS BY LIQUID CHROMATOGRAPHY- ELECTROSPRAY NEGATIVE IONIZATION TANDEM MASS SPECTROMETRY

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Chloramphenicol (CAP) is subjected to monitoring in food products, with a minimum required performance level set at 0.3 ng/g. CAP was isolated from chicken meat and seafood by very simple solvent extraction procedure. For honey, a fast SPE procedure was applied. CAP-D5 was used as internal standard. HPLC separation was done on RP18 123 x 3mm column in acetonitrile-ammonium formate 10 mM, pH 3.0 (40:60) at flow rate of 0.3 m/min. A TSQ Quantum instrument with ESI source has been used in negative ionization mode. A MRM procedure has been applied and following transitions were monitored: m/z 321>152 (quantifier), 321>194, 321>257 (qualifiers), 326>157 (IS). CAP peak was eluted at around 5 min; the total run time was 7 min. LOD was around 0.1 ng/g meat or 0.05 ng/g honey. For confirmation, ion intensity ratios were used. Matrix effects were studies using various approaches. The method allows analyzing up to 30 duplicate samples/day, including all calibration standards. Additionally, the method for determination of CAP glucuronide (CAP-G) was established. As a source of CAP-G urine specimens taken from rats which were given CAP were used. For the isolation of CAP and CAP-G a SPE procedure, used also for honey, was applied.

Keywords: Chloramphenicol, Chloramphenicol glucuronide, LC-MS-MS

BROAD-SPECTRUM BENZODIAZEPINE SCREENING BY LC-MS/MS

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Screening biological samples for benzodiazepines is often carried out using immunoassay (IA) techniques. However, due to the chemical diversity of this class of drugs, specific identification, confirmation and quantitation of the benzodiazepines present in forensic toxicology generally requires multiple analytical systems (LC, GC & GC/MS). Prior to validation of this work, confirmation of a putative positive result by IA in this laboratory necessitated the use of 11 separate analytical methods with the potential to consume 19 mL of sample. This led us to develop a single analytical technique for the screening and identification of benzodiazepines. A solid phase extraction (SPE) method coupled with LC-MS/MS analysis, was developed to simultaneously detect 14 benzodiazepines and 9 benzodiazepine metabolites in 1 mL of blood or urine. The compounds and their respective limits of detection (LOD) in blood included in this assay are alprazolam (1.5 ng/mL), α-hydroxyalprazolam (1.5 ng/mL), bromazepam (1.5 ng/mL), chlordiazepoxide (5 ng/mL), demoxepam (5 ng/mL), clobazam (3 ng/mL), clonazepam (1.5 ng/mL), 7-aminoclonazepam (3ng/mL), diazepam (20 ng/mL), nordiazepam (5 ng/mL), flunitrazepam (1.5 ng/mL), 7-aminoflunitrazepam (1.5 ng/mL), n-desmethylflunitrazepam (1.5 ng/mL), flurazepam (1.5 ng/mL), n-desalkylflurazepam (1.5 ng/mL), lorazepam (3 ng/mL), midazolam (1.5 ng/mL), nitrazepam (3 ng/mL), 7-aminonitrazepam (1.5 ng/mL), oxazepam (3 ng/mL), temazepam (20 ng/mL), triazolam (1.5 ng/mL) and α-hydroxytriazolam (1.5 ng/mL).

The extraction method utilizes Waters Oasis™ HLB extraction cartridges on a Zymark Rapidtrace SPE workstation. Samples are precipitated with acetonitrile and then diluted with pH 6 phosphate buffer prior to loading onto the extraction columns. After washing with water, phosphate buffer, and 5% methanol, the compounds are eluted with 95% methanol. The samples are subsequently derivatized with acetic anhydride. Analysis of the extracted samples is performed on a Thermo-Finnigan LC-MS/MS system. The HPLC is a Thermo-Finnigan Surveyor™ equipped with a Waters Symmetry® C18 (2.1 mm x 20 mm, 5 µm particle) guard column followed by an Agilent Zorbax SB-C18 (2.1 mm x 150 mm, 3.5 µm particle) analytical column. Separation is achieved isocratically using a 54:46 methanol:ammonium formate pH 3 buffer at a flow rate of 225 µL/min and a temperature of 35°C. The MS/MS system is a Thermo-Finnigan TSQ Quantum operated in APCI/SRM (atmospheric pressure chemical ionization/selected reaction monitoring) mode. With the exception of alprazolam, α-hydroxyalprazolam, flunitrazepam, 7-aminoflunitrazepam α-hydroxyalprazolam and lorazepam, the limit of detection for this method is significantly lower than any of the previously used target analyses utilized by our laboratory.

From July 2003 to April 2004, we have analyzed 382 case samples. 52% of these samples were positive for at least one benzodiazepine or benzodiazepine metabolite. The most commonly found benzodiazepines were clonazepam, diazepam, lorazepam, and temazepam. No case samples were positive for either flunitrazepam or its metabolites. Of the positive findings, 81% were below the limit of detection for the original target analysis used in our laboratory. While the detection of low concentrations of these compounds is not usually an essential requirement in death investigations, they can be critical in drug-facilitated sexual assault investigations. In the latter instance, sexual assaults are often associated with the ingestion of low drug doses and a long time elapse between the assault and sample collection. These factors create an analytical challenge that requires sensitive and specific methodology. The benefits of this assay will be highlighted with case examples.

Key Words: Benzodiazepines, LC-MS/MS, Screening

DETERMINATION OF STRYCHNINE IN HUMAN BLOOD USING SOLID PHASE EXTRACTION AND GC/EI-MS

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Introduction: Strychnine is the main alkaloid in *Strychnos nux vomica*, an Indian tree. This plant was first introduced in Germany in the XVI century, as a poison to rats and other pests. Although widely used in the past, even with pharmaceutical purposes, its use is now limited in many countries. Strychnine is not commercialized in Portugal since 1974, but sometimes it is still associated with forensic intoxications, because small amounts may remain in storage, particularly in rural areas. The objective of this work was the development and validation of a simple and rapid method for the determination of strychnine in human blood, employing solid phase extraction (SPE) and GC/EI-MS.

Materials and Methods: Oasis HLB (30 mg) extraction cartridges were obtained from Waters (Milford, MA, USA). Stock solutions of strychnine and papaverine (internal standard) were prepared in methanol, protected from light and stored at 4 °C until use.

The sample was submitted to the following procedure: to 500 µL of blood were added 2 mL of distilled water, and the mixture was centrifuged at 3000 rpm for 5 minutes. The supernatant was then applied to previously conditioned SPE columns. After elution of the sample the columns were washed with 1 mL of 5% methanolic solution, and then dried under full vacuum for 15 minutes. The elution was performed with 1 mL of chloroform, which was afterwards evaporated to dryness under a gentle stream of nitrogen. The dry residue was reconstituted in 50 µL of methanol, and an aliquot of 1 µL was injected in the GC.
The GC oven temperature program started at 150 °C for 1 minute, then raised by 35 °C/min to 200 °C, held for 1 minute and finally elevated by 40 °C/min to 270 °C, where it was kept for 7 minutes.
The injector port was set to 200 °C. The mass spectrometer temperature was 280 °C, and it was operated in the SIM (Selective Ion Monitoring) mode. The selected ions were 334, 120 and 162 for strychnine; and 338, 324 and 308 for papaverine.

Results: The calibration curve was established in spiked blood within a range of 0.10 to 2.50 µg/mL, and the correlation coefficient was 0.9994. The limits of detection and quantification were respectively 60.27 and 100 ng/mL. The precision (coefficient of variation), calculated at both low and high concentrations, was inferior to 10%. Accuracy was superior to 90% for all calibrators. Mean recovery for strychnine was 90.66%. The method was applied to authentic samples obtained from the Laboratories of Forensic Toxicology of the National Institute of Legal Medicine, Coimbra and Lisbon, Portugal.

Conclusion: One may conclude that the proposed technique is analytically suitable for the extraction and determination of strychnine in blood, since it is linear within the studied range, and presents adequate precision and accuracy. Therefore, it can be applied in forensic cases where the compound is involved.

Keywords: Strychnine, SPE, GC/MS

EXTENDING AND IMPROVING SCREENING AND TARGET ANALYSIS UTILIZING LC-MS

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GC/MS has long been a powerful and useful technique for analysis of complex mixtures, as well as target analyses, because of its inherent specificity and sensitivity. However, the necessity for analytes to be labile limits the types of compounds that can be analyzed utilizing this technique and also requires some extensive sample preparation, such as derivatization, for successful analysis. However, over the past 15 years, LC/MS has grown in popularity due to its applicability for analysis of polar molecules, which includes most drug and pharmaceutical compounds. LC/MS also has the capabilities to analyze higher molecular weight compounds that are not easily examined by GC/MS due to their high boiling points; derivatization is rarely, if ever, necessary. Furthermore, chromatographic analysis times are generally between 10-40 minutes for LC, which are much shorter than the average times used for GC analysis. This paper examines the utility of LC/MS to reduce total analysis times of current protocols, as well as develop methods to analyze or screen for compounds that have no current methodology in the forensics field. Several types of compounds were studied for target analysis, both quantification and general screening, covering a range from warfarins to common pesticides to neurontin. Currently, these compounds have been analyzed by either GC/MS or immunoassay. However, the utility of these methods is limited by throughput and/or cost. By using LC coupled with a triple quadrupole mass spectrometer, screening of over 50 compounds in a single analytical experiment can be accomplished with low ppb detection limits. Furthermore, the compounds can be analyzed with minimal sample preparation and short analysis times, improving the throughput and greatly reducing expense.

Key words: Method development, Mass spectrometry, LC/MS

SIMULTANEOUS DETERMINATION OF SILDENAFIL, VARDENAFIL AND TADALAFIL AS ADULTERANTS IN DIETARY SUPPLEMENTS BY LC-ESI-MS

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In recent years, there is a problem with illegally added prescription ingredients and/or their synthetic analogues (as active adulterants) in dietary supplements. Prolonged or excessive consumption of these products may cause possible serious health risks to some users. It has been reported that sildenafil (SDF), which is therapeutically used for penile erectile dysfunction, is one of the active adulterants in products advertising roborant nutrition. Moreover, its structurally similar anti-impotence drugs, vardenafil (VDF) and tadalafil (TDF), may be alternative adulterants of SDF. In this study, a simultaneous analytical procedure for three anti-impotence drugs, SDF, VDF and TDF (as adulterants in dietary supplements advertising roborant nutrition) was developed using LC-ESI-MS.

These drugs were extracted with the solvent consisted of acetonitrile and distilled water containing 1 % formic acid (4:1, v/v) under ultrasonication. The separation was achieved on an Inertsil ODS-3 column (2.1 x 150 mm, 5 □m) at 40 °C. The following gradient system was used with mobile phase A (5 mM ammonium formate buffer (pH 3.5) / acetonitrile (75:25, v/v)) and mobile phase B (acetonitrile); B: 0 % (0-3 min), B linear from 0 to 30 % (3-13 min), B: 30 % (13-30 min). The flow rate was at 0.3 mL/min. LC-MS with ESI interface in the positive ion mode was used. The linear regression of the peak area ratios versus concentration in standard solution was fitted over the concentration range of 10 – 10000 ng/mL (SDF), 5 – 5000 ng/mL (VDF) and 50 – 25000 ng/mL (TDF). All calibration curves were obtained with correlation coefficients of greater than 0.9990. The recoveries of SDF, VDF and TDF spiked a functional food at 0.01mg/mg concentrations were 98.8%, 100.2% and 99.8 %, respectively.

The developed method was applied to the determination of SDF, VDF and TDF in 91 dietary supplements obtained from the Japanese market. SDF was identified in seven samples and their contents were in the range of 24.2 mg – 120.9 mg /capsule or bottle. In addition to SDF, homosildenafil (a synthetic analogue of SDF) was also detected in one of the seven samples. TDF was detected in one sample and the content was 0.32 mg/capsule, while VDF was not found in any sample. Moreover, a new synthetic analogue of SDF, hydroxyhomosildenafil, which has never been reported, was also identified in the sample.

Keywords: Anti-impotence drugs, LC-MS, Dietary supplements

SCREENING OF CANNABINOIDS, BENZOYLECGONINE AND OPIATES IN WHOLE BLOOD AND URINE USING EMIT II PLUS IMMUNOASSAY AND KONELAB 30

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Aims: To present a full validated simple and automatic method for screening of cannabinoids, benzoylecgonine and opiates in whole blood and urine for routine use.

Methods: Blood samples were extracted with acetone before analysis. Urine samples were automatically diluted 5 times. EMIT II Plus calibrator level 1, 2, 3 and 5 were used for calibration. The analysis was done with EMIT II PLUS reagents (cannabinoids, cocaine metabolite and opiate assays) on a Konelab 30 instrument at 340 nm.

Results: Cut offs in blood and urine are respectively: cannabinoids: 0.0045 mg/kg (blood) and 0.050 mg/kg (urine); benzoylecgonine: 0.015 mg/kg (blood) and 0.300 mg/kg (urine); morphine: 0.020 mg/kg (blood) and 0.300 mg/kg (urine). Within day precisions for blood controls spiked at cut off were 5-10% (cannabinoids), 10% (benzoylecgonine) and 5-10% (morphine) (n=10). Between day precisions were 6% (cannabinoids), 18% (benzoylecgonine) and 12% (morphine) (n=7). Sensitivity was 100% (cannabinoids), 93% (benzoylecgonine) and 97% (opiates). Specificity was 86% (cannabinoids), 99% (benzoylecgonine) and 95% (opiates).

Conclusion: A validated method has been described. The method is precise, robust and useful for screening of cannabinoids, benzoylecgonine and opiates in whole blood and urine.

Keywords: Cannabinoids, Benzoylecgonine and opiates, EMIT II Plus immunoassay

FAST QUANTIFICATION OF ETHANOL IN WHOLE BLOOD SPECIMENS BY THE ENZYME ALCOHOL DEHYDROGENASE METHOD; OPTIMIZATION BY EXPERIMENTAL DESIGN

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A sensitive, fast, simple and high throughput enzymatic method for the quantification of ethanol in whole blood (blood) on Hitachi 917 is presented. Alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde using the coenzyme nicotinamide adenine dinucleotide (NAD), which is concurrently reduced to form NADH. Method development was performed with the aid of factorial design, varying pH and concentrations of NAD^+ and ADH. The linear range increased and reaction end point decreased with increasing NAD^+ concentration and pH. The method was linear in the concentration range 0.0024-0.4220 g/dl. The limit of detection (LOD) and limit of quantification (LOQ) were 0.0007 g/dl and 0.0024 g/dl, respectively. Relative standard deviations (RSD) for the repeatability and within laboratory reproducibility were in the ranges 0.7-5.7 % and 1.6-8.9 %, respectively. The correlation coefficient when compared to head space gas chromatography flame ionization detection (HS-GC-FID) methods was 0.9903. Analysis of authentic positive blood specimens gave results that were slightly lower than those of the reference method.

Key words: Ethanol, ADH, Quantitation

A RAPID METHOD FOR THE ANALYSIS OF ATOMOXETINE IN BLOOD USING GC/MS

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Atomoxetine is a selective norepinephrine reuptake inhibitor (SNRI) prescribed for the treatment of attention deficit/hyperactivity disorder (ADHD). It is the first non-stimulant drug approved by the FDA for the treatment of this disorder in both adults and children. Since its approval in November 2002, over two million prescriptions have been filled. It has been estimated that 3% of the U.S. adult population may have ADHD. A review of the current literature has identified no gas chromatographic/mass spectrometric (GC/MS) methods for the detection of atomoxetine in the forensic toxicology setting. Therefore, our laboratory developed a method for the identification and quantitation of atomoxetine in postmortem blood using GC/MS. This procedure incorporates a Varian Bond Elut® Certify solid phase extraction (SPE) followed by derivitization with pentafluoropropionic anhydride (PFPA). Derivitization of atomoxetine with PFPA, although not required for the GC/MS analysis of this compound, affords both superior linearity and sensitivity over the non-derivatized compound. The method described is highly selective and sensitive, having a limit of detection of 1 ng/mL for atomoxetine. Atomoxetine was found to have a linear dynamic range of 3 – 800 ng/mL on a calibration curve weighted by a factor of 1/x. The SPE provided an efficient sample extraction yielding recoveries of 40 ± 3% and 51 ± 4% at 25 and 250 ng/mL (n=5 for each group). Furthermore, the developed procedure provided superb accuracy and precision. This procedure showed intra-day (within day) relative errors of ≤ 5% and relative standard deviations (RSD) within 2% for both the 25 ng/mL and 250 ng/mL control groups (n=5 for each group). Using whole blood controls stored at 4°C the inter-day (between day) relative errors for the 25 ng/mL control group were 9%, 10% and 3% for days 2, 3 and 7, respectively (n=5). The relative errors for the 250 ng/mL control group were 6%, 3% and 4% for days 2, 3 and 7, respectively (n=5). The RSDs were all < 5% for both control groups over the 7-day period. Based on the day 7 results, it is clear that atomoxetine is stable in blood stored at 4°C for at least one week. The method developed proved to be rapid, reliable and sensitive for the identification and quantitation of atomoxetine in blood.

Key Words: Atomoxetine, SNRI, Forensic Toxicology

STABILITY OF PLASMA ACETALDEHYDE DETERMINED BY GAS CHROMATOGRAPHY-POSITIVE ION CHEMICAL IONIZATION-MASS SPECTROMETRY

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Simultaneous abuse of drugs and alcohol is common. Acetaldehyde is a metabolite of ethanol that accumulates when ethanol is taken with disulfiram and in many individuals with genetic variants in alcohol dehydrogenase and /or aldehyde dehydrogenase. Analysis of acetaldehyde is considerable difficult due to the volatility and various factors, such as enzymatic and nonenzymatic oxidation. Most previously published methods require immediate on-site handling of specimens to allow analysis. A goal of this work was to develop a method where samples can be collected as plasma, stored and shipped for off-site analysis. A novel method was developed for the determination of acetaldehyde in human plasma that utilizes a simple liquid-liquid extraction procedure and gas chromatography-positive ion chemical ionization-mass spectrometry (GC-PCI-MS). Using acetaldehyde-d_4 as internal standard, both acetaldehyde and acetaldehyde-d_4 were derivatized directly in plasma with 2, 4-dinitrophenylhydrazine in hexane solvent at room temperature. The derivatized- acetaldehyde and acetaldehyde-d_4 were extracted into hexane. After centrifugation, an aliquot of supernatant was transferred to an autosampler vial for analysis. A GC capillary column was used for the separation of the derivatives, which were subsequently ionized with ammonia reagent gas and analyzed by MS. The GC oven temperature was initially set at 120°C and increased to 300°C at 20°C /minute. Run time for each injection was 10 minutes. MS source and quadruple temperatures were set at 200 and 150 °C, respectively. The prominent ions at m/z 242 and 246 for acetaldehyde and acetaldehyde-d_4, respectively, were analyzed by selected ion monitoring. The lower limit of quantification was 0.3 μg/mL in 0.5 mL of plasma and the linearity in 4 assays was r^2=0.998, over a range from 0.3 to 20 μg/mL. When intra- and inter-assay precision and accuracy were evaluated at concentrations of 0.75, 8 and 15 μg/mL, mean measured concentrations did not deviate more than 14% from the target and coefficient of variance did not exceed 4%. After derivatization, the extracts of acetaldehyde and acetaldehyde-d_4 were found to be stable for up to 117 hrs in autosampler vials at room temperature. This method is simple because of: no need for protein precipitation; single step in liquid-liquid solvent extraction; no concentration steps, such as drying supernatant and reconstitution. The method was used to study the stability of acetaldehyde in human plasma. In conclusion, this simple and reliable method appears to be useful in toxicology and other researches. The research is supported by NIDA Contract N01DA-3-8829.

Key Words: Acetaldehyde, GC-MS, Stability.

MULTICENTER EVALUATION OF THE ROCHE ONLINE® TDM CARBAZEPINE ASSAY FOR ROCHE/HITACHI ANALYZER SYSTEMS

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Carbamazepine is an anticonvulsant and anti-manic drug, used in the treatment of trigeminal neuralgia, bipolar disorders, epilepsy, and a wide variety of seizure disorders. Monitoring carbamazepine concentrations is essential during therapy in order to ensure achievement of optimal therapeutic effect, while avoiding the impact of both subtherapeutic and toxic drug levels.

The analytical performance of a new, Roche homogeneous microparticle immunoassay for Carbamazepine was evaluated at four sites – two in Europe and two in the Unites States. Intra- and interassay imprecision, lower detection limit, control recovery, and linearity were assessed. Further, the assay method was compared to the CEDIA Carbamazepine assay on Roche/Hitachi 917 and MODULAR P analyzers, Abbott FPIA Carbamazepine assay on the Abbott AxSYM and TDx analyzers, and the Roche FP assay on INTEGRA 800. Serum vs. sodium heparin plasma comparison was also performed.

OnLine Carbamazepine intra-assay imprecision showed SDs ≤0.14 µg/mL for concentrations up to 4 µg/mL and CVs ≤3.4 for concentrations >4 µg/mL. Interassay imprecision showed SDs ≤0.15 µg/mL for concentrations up to 4 µg/mL and CVs ≤4.1 for concentrations >4 µg/mL. Analytical sensitivity (lower detection limit) to 0.22 µg/mL and linearity to the 20.0 µg/mL were observed. Roche COBAS FP control materials recovered within range at all sites and competitor control materials recovered within manufacturers' ranges with one exception.

Passing/Bablok regression analysis was used to assess method comparison. All comparisons demonstrated close agreement between competitive methods as noted in the table below:

	Methodology/Instrument x	y	N	Slope	Intercept	Correlation Coefficient
Site 1	CEDIA MOD P	OnLine MOD P	168	1.055	-0.248	0.993
Site 2	CEDIA 917-R	OnLine 917-R	157	0.982	0.093	0.983
Site 3	CEDIA MOD P	OnLine MOD P	161	1.059	-0.642	0.972
	COBAS FP INTEGRA 800	OnLine MOD P	161	1.121	-0.458	0.984
	Abbott AxSYM	OnLine MOD P	161	1.120	-0.426	0.975
Site 4	CEDIA 917	OnLine 917	145	1.039	-0.409	0.988
	Abbott TDx	OnLine 917	145	1.068	-0.111	0.986

Comparison of serum vs. sodium heparin plasma produced this regression equation: $y = 1.107x - 0.074$, $r=0.982$ ($n=47$). The OnLine Carbamazepine TDM reagent met or exceeded analytical specifications and all clinically relevant performance criteria in this evaluation.

Key Words: Carbamazepine, Automated Method, Multicenter Evaluation

AN LC-MS/MS METHOD FOR THE QUANTIFICATION OF BUPRENORPHINE AND NORBUPRENORPHINE IN BLOOD AND URINE

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Background: The synthetic opioid buprenorphine has become common in the maintenance therapy of opioid addicts in Finland since 1999. The increasing number of samples has required a simple and reliable analytical method suitable for routine use. LC-MS/MS provides high selectivity and sufficient sensitivity for the quantitation of buprenorphine and its metabolite norbuprenorphine in urine and blood.

Methods: Urine samples were hydrolyzed with β-glucuronidase enzyme. Sample work-up for blood and hydrolyzed urine involved liquid-liquid extraction with ethyl acetate at pH 7. The extracts were run by LC on a Genesis C_{18} reversed phase column using acetonitrile-ammonium acetate mobile phase at pH 3.2. The mass spectrometric analysis was performed with a triple quadrupole mass spectrometer equipped with a turbo ion spray interface in positive mode using multiple reaction monitoring (MRM). Sufficient sensitivity was achieved only by monitoring the surviving parent ions both for buprenorphine (*m/z* 468.0) and norbuprenorphine (*m/z* 414.2) at the collision energy of 20 eV. Quantification was performed using deuterated internal standards and four-point calibration.

Results: Validation curves were prepared with four replicates at ten concentration levels. The linearity criterion was accuracy better than ± 20% at the calibration curve. The method exhibited good linearity from 1 to 200 µg/l for urine ($r^2 \geq 0.996$, quadratic regression) and from 0.2 to 100 µg/l for blood ($r^2 \geq 0.998$, linear regression through zero). The average intra-day precision of real autopsy samples was 3 % for both compounds in urine, and 7% for buprenorphine and 11% for norbuprenorphine in blood. Criteria for the LOQ were: 1) precision better than ± 20%, 2) inaccuracy lower than ± 25% in urine and 20% in blood, and 3) signal-to-noise ≥ 10. The LOQs for both compounds were 1 µg/l in urine and 0.2 µg/l in blood. In the study of uncertainty of measurement, systematic errors were not observed. The expanded uncertainty of measurement (at the confidence level of 95%) contained only random errors being for both compounds 15% and 30% in urine and blood, respectively. A total of six proficiency testing samples (UKNEQAS 2003, Labquality 2004) containing buprenorphine were analyzed with the established method, and the results were within 11% of the consensus or average value.

Discussion: The present method has been applied to blood and urine samples from more than 420 autopsy cases and 670 living patients. Interferences from sample matrix have been detected only very rarely even though the surviving parent ions are monitored to obtain adequate selectivity. Successful participation in international proficiency testing schemes proves the high reliability of the method.

Keywords: Buprenorphine, Quantification, LC-MS/MS

VALIDATION OF AN EIA-BASED SCREENING ASSAY FOR THE DETECTION OF AMPHETAMINE AND MDMA/MDA IN BLOOD AND ORAL FLUID

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The number of seizures and the use of amphetamine and 'ecstasy' (MDMA) have increased exponentially in Belgium since the late nineties. Therefore, screening for these substances in biological specimens has become an important part of routine analysis in forensic toxicology laboratories. The use of a reliable sensitive immunological assay for the screening of blood and oral fluid samples is less widespread than for the preliminary analysis of urine. Such an assay would save cost and labor time in comparison to a more specific analysis as gas chromatography-mass spectrometry (GC-MS).

The objective of this study was to evaluate the suitability of the Cozart® AMP enzyme-linked immunoassays (EIA) for the screening of blood and plasma samples, collected with sodium fluoride and potassium oxalate as anticoagulant, and oral fluid samples, collected with the Intercept® device.

Authentic blood samples (n = 260) were assayed on the EIA plate, using an optimal 1:5-fold dilution. True positive, true negative, false positive and false negative results were determined relative to our routine GC-MS analysis. The EIA readily detects MDA but shows minimal crossreactivity with MDMA (< 0.1%), so the interpretation of the GC-MS result of the MDMA-only samples was based on the combined MDA/MDMA concentrations. Samples consisted of 100 amphetamine-only positives, 100 MDMA/MDA-only positives, and 60 negatives, using the limit of quantitation as the cut-off level for confirmation (10 ng/ml). Using these results, receiver operating curves (ROC) were generated and optimal cut-off values for the screening assay were calculated.

For the amphetamine positive samples, the analysis showed an optimal cut-off value at 66.5 ng/ml amphetamine equivalents with a sensitivity of 99.0 % and a specificity of 96.9 %. For MDMA/MDA positive samples, 97.0 % sensitivity and 96.9 % specificity were reached at the same cut-off value of 66.5 ng/ml amphetamine equivalents. The area under the ROC curve exceeded 0.97. When combining the results, the EIA assay is able to predict the presence of either amphetamine or MDMA/MDA in plasma samples with 98.0 % sensitivity and 96.9 % specificity at a cut-off value of 66.5 ng/ml amphetamine equivalents.

A similar analysis was conducted on 216 oral fluid specimens collected from a controlled double blind study. Subjects received placebo or a high (100 mg) or low (75 mg) dose of MDMA. Plasma and oral fluid samples were collected at 1.5 and 5.5 hours after administration. Preliminary analysis of the oral fluid samples indicated a screening cut-off of 51 ng/ml amphetamine equivalents with a sensitivity of 97.9 % and a specificity of 98.6 %, using the plasma data for confirmation. LC-MS/MS confirmation of the oral fluid samples is in progress.

In conclusion, these data indicate that the Cozart® AMP EIA plates constitute a fast and accurate screening technique for the identification of amphetamine and MDA/MDMA positive blood samples and oral fluid specimens collected with Intercept®.

Keywords: EIA, Amphetamine, Blood

SIMULTANEOUS ANALYSIS OF HIPPURIC ACID AND METHYLHIPPURIC ACIDS IN URINE FROM PAINT THINNER ABUSERS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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A high-performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) assay for the simultaneous analysis of hippuric acid and methylhippuric acids in urine is described. Urine samples were collected from abusers sniffing paint thinner including toluene and a small amount of xylenes. These samples were centrifuged at 3000 rpm for 10min, then diluted by distilled water 100 times and a 10ul aliquot of sample was injected into LC/MS. 1% acetic acid, methanol, THF (90:5:5, V/V) was used as a mobile phase. The method described allows separation of hippuric acid and methylhippuric acid in less than 40 minutes using a stainless steel column packed with octadecyl dimethylsilyil silica. The separation was possible for m-hippuric acid and p-methylhippuric acid. The detection limits of hippuric acid and methylhippuric acids were 0.5ug/ml (SIR mode), respectively. The calibration curves were linear in range of 1 to 1000ug/ml. Methylhippuric acids were detected from 36 cases of paint thinner abusers and were not detected in urines of 10 normal subjects.

Key words: Methylhippuric acids, LC/ESI-MS, Paint thinner abuser

LC/MS/MS AS A ROUTINE METHOD FOR ANALYSIS OF THERAPEUTIC AND ILLICIT DRUGS IN FORENSIC SCIENCE

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Introduction : The aim of this presention was to evaluate the usefulness of Liquid Chromatography coupled with Tandem Mass Spectrometry (LC/MS/MS) in routine forensic toxicological casework. Presently, Gas Chromatography coupled with Mass Spectrometry (GC/MS) is the most common method used for confirmation of drugs and its metabolites. However, this technique has the disadvantage of requiring derivatization, because some substances and metabolites are polars and thermolabiles. This drawback can be overcomed by using LC/MS/MS. LC/MS interface does not involve heat and offer gentle ionization of polar and thermolabile compounds. This technique has been used for routine determination of several groups of drugs: opiate, cocaine and its metabolites, amphetamine and other psychoactive phenethylamines, benzodiazepines derivatives, Δ^9-THC and its metabolites and cardiac glycosides.

Experimental : The basic urine or blood were extraction using a solid phase extraction (SPE). Chromatographic separation was acheived using a Symmetry (Waters) C-18 or C-8 column. Mobile phase conditions used a ratio of MeOH and buffer at a flow rate of 1 mL/min. A TSQ-7000 API2 triple quadrupole instrument equipped with a APCI ionization source was used in positive or negative mode.

Results : LC/MS/MS became a very powerful and flexible method for dedicated analyses of substances of forensic interest. It has been show that the use of fast LC/MS/MS provide excellent specificity and high sensitivity. Spiked urine and blood samples of opiate, cocaine and its metabolites, amphetamine and other psychoactive phenethylamines, benzodiazepines derivatives, Δ^9-THC and its metabolites and cardiac glycosides were analyzed in selected reaction monitoring (SRM) mode to evaluate limit of detection (LOD), limit of quantification, linearity and accuracy. LOD obtained range from 0.05 to 2 ng/mL for target coumpounds in matrices. The specificity of the method was evaluated with numerous antemortems and post-mortem matrices show no significant interferences at the expected retention time of target compounds. This technique improve the analysis of polars and thermolabile compounds and decrease analysis time.

Key Words : LC/MS/MS, Biological Matrices, Forensic

DETERMINATION OF ETHYL GLUCURONIDE IN URINE BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Ethyl glucuronide (EtG) is a metabolite of ethanol, formed by enzymatic conjugation of ethanol with glucuronic acid, and is regarded as a useful bio-marker of recent alcohol consumption. EtG can be detected in blood, urine, tissues and hair for an extended time period due to its specific properties of non-volatility, water-solubility and general stability in comparison to ethanol. A gas chromatography-tandem mass spectrometry (GC-MS/MS) method was therefore developed for the routine determination of EtG in urine. Initial extraction involves 3 ml of urine specimen being spiked with 45 ul of EtG-D5 (200 ug/ml) as internal standard and was adjusted to pH 6.0. Following extraction using a *Strata NH2* SPE column with methanol and water, the extract was evaporated and derivatized with BSTFA (in 1% TMCS and pyridine) at 80°C for 30 minutes. After evaporation, the residue was reconstituted in 100 µl of acetonitrile. EtG was assayed on a Varian Saturn 2000 ion trap GC-MS/MS. The standard curve was linear between 1000 and 10,000 µg/ml with curve correlation coefficients exceeding 0.99. The recovery of EtG from solid phase extraction was approximately 99% at 1000 µg/L and 102 % at 8,000 µg/L. Measurement of EtG in urine makes it possible to monitor ethanol consumption using a direct metabolite. This GC-MS/MS method provides a rapid, simple and specific determination of ethyl glucuronide.

Key words: Ethyl glucuronide, Gas Chromatography –Tandem Mass Spectrometry, Solid Phase Extraction

SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHIC ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES IN BIOLOGICAL SAMPLES

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A headspace solid phase microextraction (HS-SPME) method in combination with GC–NPD was developed for the determination of a mixture of organophosphorus pesticides (malathion, parathion, methyl parathion and diazinon) in biological samples. Fenithothrion was selected as the internal standard. A 85 μm polyacrylate (PA) SPME fiber was selected for sampling. Various extraction parameters were studied and optimized: salt addition, desorption time, extraction time, extraction temperature. Best conditions found were: 0.8 g NaCl added in 3 ml aqueous sample, 20 min sampling at 70 °C, 3 min desorption at 230°C. The HS-SPME method in combination with GC-NPD provided satisfactory detection sensitivity and liner dynamic range. The method was applied in the determination of the pesticides in various biological specimens: human whole blood, plasma, liver, kidney and cerebrospinal fluid. The inherent selectivity provided by the NPD detector together with the efficient sample clean-up of the HS-SPME resulted in clear chromatograms with no interferences. Extraction recovery varied significantly from specimen to specimen. Linear response data for these OPPs was obtained with correlation coefficients ranging between 0.9866 and 0.9999. High GC signals were observed for diazinon, as a result low limits of detection were observed. Limits of detection (LODs) were in the range of 2–10 ng/g for diazinon. In contrast the signals obtained for methyl parathion and malathion were significantly lower, thus higher limits of detection were observed. Estimated LODs ranged from 35 to 55 ng/g for methyl parathion. The proposed methodology renders an efficient, cost effective and simple and sample preparation process for the determination of OPPs. Most important, the technique overcomes limitations and obstacles of conventional methods such as the use of expensive and toxic organic solvents and the application of tedious and cumbersome procedures.

Keywords: Organophosphorus pesticides, HS-SPME, Biological samples

EFFECTS OF ASCORBATE DERIVATIVE ON SERUM HYDROXY RADICALS IN ETHANOL-TREATED RATS

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Objective: It is well known that acute and/or chronic ethanol (EtOH) increases toxicity in human and animals, which might be mediated through oxidative stress. The present study was designed to investigate the effects of EtOH on serum hydroxy radicals (HR). Furthermore, radical scavenge effect of L-ascorbyl-2-phosphate Na (APS) was also examined.

Materials and Methods: In order to investigate the oxidative stress caused by EtOH in blood, 1 ml/kg b.w. of 95% EtOH was administered orally to male Wistar rats under pentbarbital anesthesia, and blood sampling from heart were done 5, 10, 15, 20, 30 and 60 min after administration of EtOH. The signal intensity of serum hydroxy radicals (HR) was measured using the ESR □Electron Spin Resonance□-Spin Trap method based on the DMPO 5,5-dimethl-1-pyrroline-N-oxide□-Fenton reaction. ESR apparatus type JES-FR30 and recorded in a Krebs-Henselelt buffer containing 100 mM DMPO. APS or saline was then administered to EtOH-treated rats, and the signal intensity of serum HR was measured in the same manner in an attempt to determine their suppressive effects on EtOH-induced oxidative stress.

Result: EtOH significantly increased the signal intensity of serum HR about twice as compared with saline ($P<0.01$, as analyzed by Student's t-test). Administration of APS significantly suppressed the increases in HR caused by EtOH ($P<0.01$, as analyzed by Student's t-test), whereas, saline did not suppress increases in HR.

Conclusion: Present study showed that acute EtOH administration thus increased HR as detected by the DMPO-Fenton reaction, and APS administration suppressed HR to levels comparable to those without EtOH in serum. Therefore, our findings suggest that oral administration of APS, an ascorbate derivative, ameliorates oxidative stress in serum, especially under the exposure of EtOH.

Keyword: Ehanol, Hydroxy radical, L-Ascorbyl-2-phosphate

AUTOMATED SOLID PHASE EXTRACTION PROCEDURE FOR THE SIMULTANEOUS ANALYSIS OF DELTA-9-TETRAHYDROCANNABINOL (THC), 11-HYDROXY-DELTA-9-TETRAHYDROCANNABINOL (11-OH-THC), AND 11-NOR-DELTA-9-TETRAHYDRO-CANNABINOL-9-CARBOXYLIC ACID (THCC) IN BLOOD, PLASMA, AND ORAL FLUID BY GC/MS/MS

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A forensic automated procedure for the quantitative determination of THC, 11-OH-THC, and THCC in whole blood, plasma, and oral fluid by GC/MS/MS is presented. Samples are treated with acetonitrile, acidified with dilute acid, and all three analytes are simultaneously extracted by solid phase extraction using the Caliper Life Sciences Zymark RapidTrace® Solid Phase Extraction Workstation with Varian SPEC® C-18 SPE cartridges. The RapidTrace® conditioned the SPE cartridges with methanol, added the sample, washed the cartridges with water/methanol, dried the cartridges with nitrogen, and then eluted the three cannabinoids with hexane/ethyl acetate. The final extracts were evaporated, derivatized with BSTFA with 1% TMCS, and analyzed by GC/MS/MS using the Varian 1200 Quadrupole GC/MS/MS System. Quantitation was accomplished using THC-d3 and THCC-d3 as internal standards. Two product ions were chosen for each analyte with one product ion serving as the qualifier ion. Retention time had to be within 1% and the ion ratio of the qualifier product ion within 20% for identification of the analytes. The limit of quantitation (LOQ) for THC and 11-OH-THC is 1 ng/mL while the LOQ for THCC is 5 ng/mL. The limit of detection (LOD) for THC and 11-OH-THC is 0.5 ng/mL, and 2.5 ng/mL for THCC. Both THC and 11-OH-THC are linear to at least 40 ng/mL while THCC is linear to at least 200 ng/mL. The recovery of all three analytes was approximately 50% for all three specimen types tested. At the LOQ for all three analytes, between-run precision resulted in a relative standard deviation of less then 12%.

Key words: THC, Automated, GC/MS/MS

ELISA FOR THE SCREENING OF OPIATES, BENZODIAZEPINES, CANNABINOIDS, METHAMPHETAMINE, AND COCAINE METABOLITE IN POSTMORTEM FORENSIC WHOLE BLOOD

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In both forensic and clinical toxicology, enzyme-linked immunosorbent assay (ELISA) based screening methods are becoming increasingly popular and replacing radioimmunoassay (RIA) methods for numerous reasons including: cost, automation, ease of use, and disposal issues. Currently, the Milwaukee County Medical Examiner's Office uses the Immunalysis RIA kits to screen whole blood specimens for common classes of drugs such as opiates, benzodiazepines, cannabinoids, methamphetamine, and cocaine metabolite. In this study, the assay performance of the RIA kits was compared to the ELISA kits from the same manufacturer using fifty-one forensic samples. To further validate the ELISA-based assays, the intra- and interday precision, cross-reactivity, and dose-response curves were analyzed for opiates, benzodiazepines, cannabinoids, methamphetamine and cocaine metabolite to determine if the ELISA kits were an acceptable alternative to the RIA kits.

Of the fifty-one samples screened by both RIA and ELISA for opiates, benzodiazepines, cannabinoids (THC), methamphetamine and cocaine metabolite, there were a total of five discordant results (3 THC, and 2 methamphetamine). Gas chromatography-mass spectrometry analysis indicated five unconfirmed positive results (false-positives) by RIA (3 cases for THC and 2 cases for methamphetamine). On the other hand, the ELISA kits had a 100% positive and negative predictive value for all five assays. The intraday coefficient of variation (CVs; $n = 10$) near the immunoassay cutoff concentration (60 ng/mL) was 4.0-7.7% for all five assays, while the interday CVs ($n = 5$) at the immunoassay cutoff concentration (50 ng/mL) was 8.4-10.7%.
Overall, a comparative assessment of common drug screening assays by RIA and ELISA from the same manufacturer indicated some differences in analytical performance. The ELISA based screening kits offered acceptable precision, superior specificity/sensitivity and was an economical alternative to the RIA kits. The ELISA-based assays are particularly attractive for forensic specimens for several reasons including: the ability to use a variety of sample matrices (e.g. whole blood), small sample volumes reducing interferences from some forensic matrices, a long shelf life, and the ability to incorporate automation and increase throughput. Based on these criteria, the Milwaukee County Medical Examiner's Office has adopted the ELISA-based assays to screen forensic samples for drugs of abuse. All presumptive positive immunoassay results are then confirmed by GC/MS.

Key words: ELISA, RIA, Method validation

THE DETERMINATION OF FORENSIC BLOOD ALCOHOLS WITH AN INERT AUTOMATED HEADSPACE SAMPLER

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Blood alcohol analysis is a widely used high-throughput application in forensic laboratories. The use of static headspace sampling has many well know advantages for determination of volatiles in a variety of less than ideal matrices. Blood or other biological fluids are certainly not the cleanest of matrices and therefore are well suited for headspace sampling. In terms of GC analysis, reduced inlet and column maintenance, better quantitation, and increased throughput are some of the advantages of automated headspace. Dual column systems offer an advantage in that the elution order of ethanol and some other common metabolites differ on the DB-ALC1 and DB-BAC2 stationary phases. This provides added confirmation and a potential reduction in possible inferences or coelutions with ethanol.

A new automated headspace sampler with 70-sample tray and inert flow path is introduced for the determination of forensic blood alcohols. The headspace sampler employs a completely inert flow path, uniform heated zones, and unique vent line purging capability. When taken together, these important attributes lead to a reduction in carryover and improved repeatability. Standard mixtures in water were used to demonstrate the analyses. Two headspace-based solutions, based on 0.53mm ID and 0.32mm ID columns, are detailed. Isothermal analyses with cycle times below 5 min are easily achieved with sufficient resolution to avoid common interferences. Total system control from the GC ChemStation is possible with 21 CFR Part 11 compliant software specific for headspace sampling.

Key words: Blood Alcohols, Headspace, Gas chromatography

DETERMINATION OF ACONITINE ALKALOIDS BY HPLC/TOF-MS: COMPARISON OF ELECTRO-SPRAY AND LASER-SPRAY IONIZATIONS

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Background: Aconitum species (Ranunculaceae) are widely distributed in Northern Asia and North America. Their roots are popularly used in herbal medicines in China and Japan; however, their diesterditerpene-type alkaloids are extremely toxic. For example, the LD50 values of aconitine in mice are 1.8 mg/kg (orally) and 0.12 mg/kg (intravenously); the estimated minimal lethal dose in man is about 2 mg. Accidental poisoning cases by aconite usually happen in Japan. Also, it is sometimes used in suicidal and homicidal cases, because of its high toxicity. It is thus important to determine aconitine alkaloids in body fluids with high sensitivity. We have succeeded in determining aconitine and its related compounds by HPLC/time-of-flight (TOF)-mass spectrometry (MS). Also, we have tried to detect aconitine alkaloids by laser-spray ionization and compared its sensitivity with that by conventional electro-spray ionization.

Methods: Aconitine alkaloids (aconitine, mesaconitine, hypaconitine, and jesaconitine) and methyllycaconitine (internal standard; IS) spiked in plasma samples were purified using a BondElut Certify HF column. Extracted and dried aconitine alkaloids are reconstituted with 50μL of mobile phase, which consists of 70 % of 20mM ammonium acetate (solvent A) and 30 % of acetonitrile (solvent B). HPLC was employed in the isocratic mode (A:B 70:30) at flow rate of 0.2mL/min. MS was performed on a JEOL (Tokyo, Japan) AccuTOF HPLC-TOF mass spectrometer; the ionization used was electro-spray ionization (ESI) in positive ion mode. The MS conditions were as follows: spray voltage; 2.2kV, orifice voltage; 75V (for quantitation) or 135V (for identification), nebulizing gas (nitrogen); 10L/min. For the laser-spray ionization, a 10 W infrared laser was used.

Results: All aconitine alkaloids and IS are completely separated on chromatogram. In human plasma samples, the calibration curves gave good linearity in the range of 5 and 300 ng/mL in all the compounds; their detection limits were about 0.3 to 1 ng/mL. Their limits for identification were about 0.7 to 2 ng/mL. The intraday CV values were less than 21 % for 200 ng/mL. In the laser-spray ionization, the ion current intensity was about 5 times higher compared to that by electron-spray ionization.

Conclusion: The calibration curves gave good linearity in the range of 5 and 300 ng/mL; aconitine alkaloids can be sufficiently detectable at toxic concentrations. Thus this method is useful for quantitation and identification of aconitine alkaloids. Laser spray ionization could be also applied to identify trace amounts of aconitine alkaloids.

Keywords: Aconitine, Mass spectrometry, Laser spray

THE DETERMINATION OF ETHYL GLUCURONIDE IN URINE USING REVERSED-PHASE HPLC AND PULSED ELECTROCHEMICAL DETECTION

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There is a need for a method to distinguish between ethanol levels detected in biological matrices due to alcohol consumption versus ethanol production after death as a result of decomposition. Ethyl glucuronide (EtG) is a non-volatile, water-soluble, direct metabolite of ethanol that can serve as a biological marker of alcohol consumption. This metabolite can be a marker of acute alcohol consumption even at low levels unlike traditional biomarkers. It is an intermediate marker of alcohol consumption, bridging the gap between long-term (CDT, MCV & GGT) and very short-term (ethanol & HTOL) biomarkers. EtG is a highly sensitive and specific alcohol consumption marker that can be detected for up to 80 hours after complete alcohol elimination from the body. This has very important clinical and forensic applications. Clinical applications for this biological marker would include monitoring patients in treatment for alcohol abuse. This marker could improve therapy outcome and quality of life in patients by preventing relapse episodes. Furthermore monitoring would increase safety in the workplace and prevent traffic accidents. Finally, fetal alcohol syndrome could be avoided by detecting and monitoring EtG in the mother which would reduce costs by making therapy more effective. In addition, the metabolite exhibits very high storage stability which is important as forensic samples are often stored for extended periods of time. Sometimes samples are not analyzed until the case goes to court which could take months or years. In previous studies there have been various methods used to detect EtG. These include gas chromatography (GC) coupled with mass spectrometry (MS), and liquid chromatography (LC) coupled with MS. GC/MS is available at almost all forensic facilities at a moderate cost. However, GC-MS detection of this metabolite requires prior derivatization. LC/MS is advantageous because it doesn't require derivatization nonetheless it is an expensive technique. For widespread use of EtG as a marker, simpler and less expensive methods are necessary.

The current study involved developing a method for the detection of EtG in postmortem urine samples using reversed-phase liquid chromatography with pulsed electrochemical detection. Methyl glucuronide served as the internal standard. The mobile phase consisted of 1% acetic acid/water and acetonitrile (98:2), with a 600mM sodium hydroxide post-column system attached to enable pulsed electrochemical detection (PED). This amperometric detection technique applies alternated positive and negative potential pulses at a noble metal electrode. The analyte is oxidized followed by oxidative and reductive cleaning steps. The analyte concentration is determined by measuring the electric current resulting from the molecule gaining or losing electrons. In order to separate EtG from the biological matrix a solid-phase extraction (SPE) was used using aminopropyl columns. EtG was found to have a retention time of 5.3 minutes with LOQ and LOD values of 0.4 and 0.1 ug/mL respectively. The extraction recovery following SPE was approximately 50%. This method is specific, reproducible and sensitive. Reversed-phase chromatography enabled a simple separation of the analyte without requiring the ion-pairing reagents typically associated with ion chromatography. PED is a direct (no derivatization) and affordable detection method. This method is a potential tool to clinical and forensic toxicologists for determining alcohol consumption in live and deceased individuals.

Keywords: Alcohol, Biomarker, Direct and affordable analysis

SIMULTANEOUS ANALYSIS FOR PSYCHOTROPIC TRYPTAMINES AND PHENETHYLAMINES USING GC-MS AND LC-ESI-MS

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Many analogs of tryptamines and phenethylamines are hallucinogenic substances that exist naturally in some plants, fungi and animals, but can also be produced synthetically. Some of these drugs are strictly controlled by the Narcotics and Psychotrophics Control Law in Japan but many non-controlled analogs have been widely distributed as easily available psychotropic substances, especially via the Internet.

To investigate the trend of these non-controlled drugs of abuse, simultaneous analytical methods were developed using GC-MS and LC-ESI-MS for 9 tryptamines/carbolines (AMT, 5-MeO-AMT, 5-MeO-DMT, 5-MeO-DIPT, 4-OH-DIPT, 4-acetoxy-DIPT, bufotenine, harmine and harmaline), 5 phenethylamines (2C-I, 2C-T-2, 2C-T-4 and 2C-T-7, including MBDB) of typically non-controlled substances in Japan, and, additionally, 4 legally controlled tryptamines and phenethylamines originally found in fungi or plants (DMT, Psylocin, Psylocybin and mescaline). Moreover, the proposed methods were applied to analyses of these drugs in 99 products (purchased at video shops or via the Internet over the past 2 years in Japan), which advertised psychotrophic/psychoactive effects.

The samples (powder, tablets or liquid) were extracted with methanol under ultrasonication. After centrifugation, the extracts were filtered thorough a 0.45-□m membrane filter prior to the injection. GC-MS analysis was performed within 30 minutes using a DB-5MS capillary column (0.25 mm i.d. x 30 m, 0.25 □m film thickness). Regarding the LC-ESI-MS analysis, the separation of the target drugs was optimized on an ODS column (Atlantis dC18, 2.1 x 150 mm, 5 □m) in an acetonitrile-10mM ammonium formate buffer (pH 3.0) by a linear gradient program and a quantitative analysis was carried out by the monitoring of each $[M+H]^+$ in the positive ion mode of ESI-MS.

As a result of the analyses using GC-MS and LC-ESI-MS, 5-MeO-DIPT (the synthetic substance known by the street name "Foxy") was found in 7 out of the 99 products except the products sold as "chemical reagents", and AMT, 5-MeO-DMT and harmine were also found in some of the 99 products. These analytical methods will be useful for the investigation of the distribution of the non-controlled psychotropic tryptamines and phenethylamines in the market.

Keywords; Tryptamines, Phenethylamines, Non-controlled psychotropic substances

A FULLY AUTOMATED METHOD FOR THE SCREENING OF BASIS DRUGS IN WHOLE BLOOD THROUGH ON LINE SPE-HPLC-DAD

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One of the major tasks in forensic toxicology laboratories, especially those with high workloads, is to provide adequate drug screening analysis in a limited amount of time. The purpose of this work was to develop a screening method, with reasonable discrimination capabilities, that could work unattended, using an aumomated device for solid phase extraction (SPE) coupled to a HPLC-DAD. Postmortem blood samples, (free of drugs) were spiked with cocaine, benzoylecgonine, lignocaine, diazepam, nordazepam, fluoxetine and sertraline, until final concentrations of 500 ng/mL were achieved. The whole blood samples were sonicated, than diluted from 3.5 to 7 mL with carbonate buffer (pH 9.3). The diluted samples were centrifuged and the supernadant was used for SPE. The extraction was performed in an Aspec® system, using Speed® C18 200mg cartridges, with the following steps: conditioning with 2 mL of methanol and 2 mL of carbonate buffer pH 9.3, loading of 6.5 mL of diluted samples, washing with 2 mL of 9:1 carbonate buffer:acetonitrile and eluting with 1 mL of 9:1 acetonitrile:phosphate buffer pH 2.3. The solvents flows and air pushes were optimized. The eluate was injected on line in the HPLC, using a 100 µL loop. For the hplc analysis, a C8 column, 25cm x 4.6 mm, was used in isocratic mode, with a mobile phase composed of phosphate buffer and acetonitrile (63:37). The identification of the drugs was made based on their relative retention times to imipramine (I.S.) and on a spectral library. Recoveries above 80% were obtained for all drugs tested, with extracts sufficiently clean for hplc-dad identification. The method is now being expanded to a bigger number of drugs, including a acidic fraction.

Key words: Solid phase extraction, Drug screening, HPLC-DAD

SOLID-PHASE MICROEXTRACTION BASED APPROACH FOR ENANTIOMERIC ANALYSIS OF AMPHETAMINES

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As a solventless approach and with recent advances in the fiber manufacturing technology, solid-phase microextraction (SPME) is now widely studied for its effectiveness for the pretreatment of various categories of samples. This study explores a novel SPME approach for enantiomeric analysis of amphetamines, in which absorption and derivatization are accomplished in one step. Specifically, (S)-(-)-*N*-(Trifluoroacetyl)-prolyl chloride was adapted as the chiral derivatizing reagent and added directly into the sample matrix. Analytical parameters, such as temperature, absorption/desorption duration, and the amount of derivatizing reagent, were studied to determine their effects on the yields of analytes on the fiber. The derivatization products resulting from this study show excellent desorption characteristics on the polydimethylsiloxane-coated fiber adapted for this study. For example, a one-time 5-min desorption leaves no detectable carry-over. Optimal operational parameters (absorption: 70 °C for 10 minutes; injection: 250 °C for 5 minutes) cause minimal negative impact on the fiber, allowing repeated used of the fiber for more than 30 times. This method was evaluated and proved to be effective in (a) quantitative determination of the enantiomeric pairs of amphetamine and methamphetamine, in terms of repeatability, linearity, and limits of detection and quantitation; and (b) generating case-specimen data comparable to those derived from a conventional liquid-liquid extraction approach (Table 1).

Table 1. Comparison of enantiomeric composition data resulting from two methods (one-step solventless SPME vs. conventional two-step liquid-liquid extraction/derivatization)

Sample	SPME (concentration in ng/mL)				Liquid-liquid (concentration in ng/mL)			
	d-Methamp.	*l*-Methamp.	*d*-Amp.	*l*-Amp.	*d*-Methamp.	*l*-Methamp.	*d*-Amp.	*l*-Amp.
1	12,169	1,280	2,477	72	10,638	1,564	2,702	63
2	1,821	219	1,010	42	1,427	146	976	32
3	7,436	593	1,329	36	7,768	429	1,318	37
4	13,845	1,960	2,581	94	11,753	1,233	2,545	67
5	3,766	340	2,675	93	3,310	252	2,763	72
6	10,745	1,254	3,324	92	9,931	1,315	2,973	79
7	3,090	704	1,466	78	2,564	591	1,381	79
8	2,482	5,863	580	403	2,010	6,317	482	538
9	5,398	486	1,971	50	4,680	435	1,928	43
10	6,667	608	2,376	84	5,649	543	2,230	66
11	6,761	947	2,220	78	5,632	449	2,100	73
12	8,600	972	1,308	90	6,276	950	1,276	58

Keywords: Amphetamines, Enantiomer, GC-MS

GC-MS QUANTITATION OF CODEINE, MORPHINE, 6-ACETYLMORPHINE, HYDROCODONE, HYDROMORPHONE, OXYCODONE, AND OXYMORPHONE IN BLOOD

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A method is described for the simultaneous analysis of seven opiates - codeine, morphine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone and oxymorphone in blood samples by gas chromatography - mass spectrometry. One milliliter of blood is combined with an internal standard mixture containing 200 nanograms of each of the seven deuterated opiates. Two milliliters of acetonitrile are added to precipitate the proteins and cellular material. After centrifugation, the clear supernatant is removed and the acetonitrile is evaporated. The remaining aqueous portion is adjusted to pH 9 with sodium bicarbonate buffer from which the drugs are extracted into chloroform / trifluoroethanol (10:1). The organic extractant is transferred and dried under nitrogen. The residue is reconstituted in dilute hydrochloric acid and washed consecutively with hexane, then chloroform. The purified aqueous portion is adjusted to pH 9 with bicarbonate buffer and the drugs are again extracted into chloroform / trifluoroethanol (10:1). The organic portion is removed from the aqueous fraction and dried under nitrogen. The residue is consecutively derivatized with methoxyamine, then propionic anhydride using pyridine as a catalyst. The ketone groups on hydrocodone, hydromorphone, oxycodone and oxymorphone are converted to methoximes. Hydroxyl groups present at the C-3 and C-6 positions of codeine, morphine, 6-acetylmorphine, hydromorphone and oxymorphone are converted to their respective propionyl esters. After a post derivatization purification step, the extracts are analyzed by full scan gas chromatography - mass spectrometry using electron impact ionization.

The method is linear to at least 2000 ng/mL. Day-to-day precision (N = 15) at 500 ng/mL and 75 ng/mL were less than 10% for all 7 targeted opiates. Extraction efficiencies at these two concentrations ranged from 50% to 68%. For each opiate, the limit of quantitation was 10 ng/mL, while the limit of detection was 2 ng/mL.

Keywords: Opiates, Blood, Mass Spectrometry

A STUDY OF CROSS-REACTIVITY OF SELECTED ANIMAL PROTEINS ON AN ENZYME-LINKED IMMUNOASSAY FOR RECOMBINANT HUMAN ERYTHROPOIETIN

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The FDA approved recombinant human erythropoietin, otherwise known as rhuEPO, for human use in 1991. Recombinant human erythropoietin was created for patients with anemia to boost red blood cell production, termed erythropoiesis. Alleged abuse due to availability during clinical trials has been reported as early as 1987 in human athletes. Administration of rhuEPO to a healthy patient is similar to blood doping or training at high altitudes. It increases the level of circulating erythrocytes, increasing the oxygen carrying capacity of the blood to reduce fatigue in the athlete competing in endurance sports. This abuse, not limited to human athletes, has been reported in horseracing as well. The unique characteristic of administration of rhuEPO to the equine athlete results in an immune reaction to the drug. This results in an autoimmune reaction to the horse's intrinsic erythropoietin, causing severe anemia and in some cases death. These reactions create antibodies detectable in an enzyme-linked immunoassay (ELISA). Questions have been proposed as to the specificity of this assay to react only to the anti- rhuEPO, and not to other intrinsic proteins in equine serum. In order to ensure the assay is detecting only the horse's antibody to rhuEPO, we have tested the assay against various immunoglobulins and sera from equine and other sources. Reactions from all of the lgG's and sera tested and potential cross reactivity between the ELISA rhuEPO plate and serum proteins in animals are presented.

Keywords: rhuEPO, Blood doping, ELISA

DETERMINATION OF SODIUM AZIDE IN BODY FLUIDS AND BEVERAGES BY ION CHROMATOGRAPHY

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Background: Sodium azide, which is widely used as a bactericide in protein samples in clinical or research laboratories, is highly toxic. Actually, its oral LD50 value is estimated to be 45 mg/kg, which is only 5 times greater than that potassium cyanide. In 1998, several mass poisoning cases with sodium azide occurred in Japan. It was then regulated as a poison by the Poisonous and Deleterious Substances Control Law. It is thus important to determine azide in body fluids and beverages with high sensitivity. We have succeeded in determining azide in different materials by ion chromatography.

Methods: Azide ion in various samples was extracted using a Conway microdiffusion cell. Hydrazoic acid was vaporized from 1 mL sample (plasma or beverages) by adding 1 mL of 5% sulfuric acid in the outer groove, and absorbed in 250 μL of 0.1 M NaOH solution in the central round basin. After the cell was incubated at 37 °C for 30 min, a 20-μL aliquot of 0.1 M NaOH was injected to an ion chromatography system equipped with a suppressor and a conductivity detector (Dionex DX 500 system). The guard and separation columns used were a Dionex AG15 (50 x 2 mm i.d.) and an AS 15 (250 x 2 mm i.d.) column. The mobile phase was 38 mM NaOH and the flow rate was set at 0.4 mL/min.

Results: The retention time of azide ion was about 10 min. In human plasma samples, the calibration curve gave good linearity in the range of 50 ng/mL and 10 μg/mL; its detection limit was about 30 ng/mL. The intraday and interday CV values for 5 μg/mL plasma were 2.8 and 8.8 %, respectively. We have tried several internal standards, but the use of an internal standard did not improve the reproducibility in quantitation of azide. Also, similar experiments were performed in different beverage samples spiked with sodium azide. It gave good linearity in the range of 50 ng/mL and 5 μg/mL; its detection limit was about 10 ng/mL.

Conclusion: Combination of the microdiffusion method and a semi-microcolumn has enabled the detection of azide with 3 to 4 times higher sensitivity compared to the methods previously reported. Thus, the present method can be applicable for clinical and forensic toxicology, because of its simplicity and sensitivity.

Keywords: Azide, Ion chromatography, Microdiffusion method

A GENERAL SCREENING AND CONFIRMATION APPROACH TO THE ANALYSIS OF DESIGNER TRYPTAMINES AND PHENETHYLAMINES IN BLOOD AND URINE USING GC/EI-MS AND HPLC/ELECTROSPRAY-MS

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Recent additions of designer tryptamines and phenethylamines to the Drug Enforcement Administration's list of controlled substances necessitate analytical procedures for their detection and quantitation. As specific immunoassays are not currently available and cross-reactivities with existing assays are unknown, a screening method based on gas chromatography mass spectrometry was developed. The method was capable of measuring the pentafluoropropionic derivative of α-methyltryptamine (AMT), N,N-dimethyltryptamine (DMT), 4-bromo-2, 5-dimethoxy-β-phenethylamine (2CB), N, N-dipropyltryptamine (DPT), 2,5-dimethyl-4-N-propylthio-β-phenethylamine (2C-T-7), and 5-methoxy-N, N-diisopropyltryptamine (5-Me-DiPT). Separation was optimized to allow tentative identification of metabolites, which display common electron impact ionization fragmentation patterns. The screening method gave limits of detection between 5-10 ng/mL and demonstrated linearity between 50-1000 ng/mL. The method was successfully applied to blood and urine samples in suspected AMT intoxications. Confirmation of 5-Me-DiPT in one of the subjects' urine was achieved using liquid chromatography/mass spectrometry (LC/MS). Quantitation by selected ion monitoring (SIM) yielded a urinary concentration of 229 ng/mL. The method was linear from 25 – 1500 ng/mL with a correlation coefficient of 0.995. The limit of detection was 5 ng/mL in urine on the LC/MS. Two additional peaks were observed and presumed to be metabolic products reported previously as 5-methoxy-N-isopropyltryptamine and 5-methoxy-N, N-diisopropyltryptamine-N'-oxide.

Keywords: Tryptamine, Phenethylamines, HPLC/ES-MS

MULTI TARGET SCREENING (MTS) FOR 300 DRUGS USING A LINEAR ION TRAP MASS SPECTROMETER AND LIBRARY SEARCHING

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A Multi Target Screening (MTS) procedure for drugs in blood and urine for toxicological analysis has been developed for the rapid detection and identification of tranquilizers (benzodiazepines), hypnotics, drugs of abuse (opiates, cocaine, amphetamines, cannabinoids), antidepressants, neuroleptics, and cardiac drugs using LC/MS/MS with a linear ion trap-based tandem-mass spectrometer. In comparison to common GC/MS or LC/MS/MS methods used for target analysis, the combination of Multiple Reaction Monitoring (MRM) and Enhanced Product Ion (EPI) scan in an Information Dependent Acquisition (IDA) experiment with library searching offers both sensitive target analyte detection and subsequent identification by a mass spectrometric database in a single LC/MS/MS run. Liquid-Liquid Extraction or Solid Phase Extraction has been used for sample clean up. Reversed-phase gradient LC with run-time of 30 minutes and MS/MS analysis have been performed in MRM using a Q TRAP™ LC/MS/MS system with a TurboIonSpray® source. Similar to previous work of Gergov et al. [1] – and in contrast to a method presented by Marquet et al. [2] , who used scan-mode for the survey scan - in the presented method 300 MRM-transitions have been monitored with short dwell times of 5 milliseconds each as survey scan of the IDA experiment. In case of a positive detection of an MRM transition, an EPI scan of the precursor ion was triggered as dependent scan, yielding product ion mass spectra at three pre-selected collision energies with the high sensitivity of the linear ion trap. Obtained EPI spectra were then searched against a newly created product ion spectral library. Fit, Reverse fit and Purity fit values are given as results in a library search hit list. A ready-to-use acquisition method and mass spectral library with Enhanced Product Ion spectra of 300 compounds is presented. The developed method was successfully applied to samples of forensic cases. Dynamic exclusion of detected MRM transitions was used to detect co-eluting compounds. This was especially important for intoxication cases, where concentrations are usually 10 to more than 100 times higher than normal therapeutic concentrations. Examples of library searching with identification based on fit and purity fit values are given for amitriptyline (at CE of 35eV) and dibenzepine (CE of 50eV) and others. Purity fit values above 0.9 were obtained for all cases. The results were confirmed by established chromatographic screening methods. The developed method showed high sensitivity and selectivity. A further advantage of this method in contrast to the published method of Gergov at al. [1] is that the sample has to be injected only once, so less time is needed for the analysis. Conclusions: The developed method is useful for the rapid detection and identification of 300 pre-selected target compounds in blood extracts of forensic and clinical cases using ESI or APCI. After introduction of retention time windows ("periods") with each containing up to 300 compounds the number of target analytes can even be increased to more than 1000 compounds per analysis.

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Keywords: Multi target screening, LC-MS/MS library, Information dependent acquisition

FORENSIC DRUG ANALYSIS WITHOUT PRIMARY REFERENCE STANDARDS

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Previously, primary reference standards have been considered essential for substance identification and a prerequisite for quantitative analysis. However, standards for designer drugs, metabolites or rare substances cannot be obtained in a reasonable period of time to allow a prompt forensic investigation. This paper reports a new approach in forensic sciences to characterize drugs without the immediate need of primary reference standards.

By liquid chromatography – time-of-flight mass spectrometry (LC-TOFMS) or Fourier transform mass spectrometry (LC-FTMS), it is possible to measure exact molecular masses on a routine basis. Especially benchtop LC-TOFMS instruments, being more cost-effective, are suitable for high-throughput work. Modern LC-TOFMS gives a high mass accuracy (approximately 5 ppm) and a moderately high mass resolution (5000-10000 FWHM). A target library consisting of exact monoisotopic masses for thousands of drugs and metabolites can be created in-house to determine the elemental formulas of sample components. This kind of mass list is easily updated by typing recent data from the literature. Even complex biological samples can be analysed without complete chromatographic separation of the components.

Chemiluminescence nitrogen detector (CLND) possesses an equimolar response to nitrogen, and consequently it provides a universal means for quantification of nitrogen containing compounds using a single secondary standard. As approximately 90% of drugs contain nitrogen, CLND is an attractive tool in forensic sciences. However, the technique has not been used in this area thus far.

In this paper, various critical aspects of LC-TOFMS analysis are discussed. These include internal mass scale calibration, dynamic range, sensitivity, library search options, isotope pattern analysis, and utilization of urine metabolic patterns. CLND analysis is discussed in terms of sensitivity, linearity, equimolarity, and general performance. The merits of LC-TOFMS identification and CLND quantification are demonstrated in instances, where the availability of standards is a problem, such as in the analysis of street drugs and in the determination of a plasma parent drug/metabolite ratio.

Keywords: Mass spectrometry, CLND, Drug analysis

FAST SCREENING AND IDENTIFICATION OF DRUGS USING RETENTION TIME LOCKING/RESULTS SCREENER BY GC-MS

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A major challenge for all forensic toxicology laboratories is to isolate, detect, identify and quantify the wide array of toxicants or drugs that may be present in a case where the cause of death is unknown. It is a challenge to screen, in a single analysis, hundreds of the most common toxicants that could be present. However, the use of a retention time locking process with GC/MS analysis affords this opportunity. This very powerful tool allows for the automated, rapid screening and identification of multiple analytes in just one injection. This tool combines the two key characteristics for the positive identification of a compound: retention time and mass spectra. After maintenance of the chromatographic system (i.e. cutting or changing the column), the retention times of the analytes can drift, which makes the positive identification of analytes more time-consuming. Furthermore, although two or more instruments may be running under the same conditions, the retention times of those analytes may not be the same. However, when using retention time locking, the inlet pressure is adjusted and the methods are locked to that pressure, resulting in consistent retention times of all compounds from run to run. With the use of this technique the event times of the method will stay the same, allowing the comparison of real time data with previously acquired data. Also the chromatograms can be superimposed, leading to easy observation and comparison of patterns. Retention time locking is used then in conjunction with a results screener, where a database is created for the locked methods. Results screener contains information of the target and qualifier ions of the analytes of interest, with their relative intensities, at their expected retention times. Therefore, every injection is made using a locked method containing a previously created database, and the system scans for the ions produced associated with an analyte in a window at its expected retention time. This allows for rapid identification of all compounds, including coeluting analytes. This detection and identification of the compounds in the sample, based on retention time and mass spectra, is achieved automatically in just a few seconds. The information obtained is then reviewed by the analyst and summarized in a customized report. Having no limits in the number of compounds that can be included in the database, retention time locking / results screener is a very powerful automated tool for the fast screening and identification of compounds in a sample. The use of this tool will be demonstrated as it is utilized for the routine analysis of toxicology samples.

Keywords: Retention time locking; GC-MS drug screening; Drug identification

USE OF STANDARD ADDITION/STANDARD DILUTION FOR QUANTITATION OF TOXICANTS IN UNUSUAL MATRICES

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At the core of forensic toxicology is the desire to quantitate toxicants from a vast array of biological matrices. Often a forensic case involves unusual matrices, including decomposed or embalmed tissues, which present complexity to the sample preparation and analysis. Analyte recovery can be variable in these types of samples; therefore, alternate approaches to quantitation must be considered. One approach taken is to construct a matrix matched calibration curve. However, blank matrix is often not readily available, and particularly by the very nature of embalmed or decomposed tissues, a true matrix match does not exist. Furthermore, recoveries from the blank matrix and actual case samples may still differ. The standard addition/standard dilution (SASD) technique, on the other hand, accounts for variable percent recovery, matrix effects, and loss of sample, while the sample itself serves as its own quality control. The SASD technique can take any of several approaches. The simplest approach involves establishing a relationship between the responses of the analyte in the native sample (blank) with the response of the native sample with a known analyte concentration added (spike), without consideration of an internal standard in the event one is not available. A second approach includes the use of an internal standard, ideally an isotopic analog of the analyte, and establishing the relationship between the response ratios of both the blank and the spike (at one or more concentrations). In a third approach, the native sample is divided into four aliquots. One aliquot is left untreated and the others are either spiked with various analyte amounts or diluted. Each sample is then assayed through the analytical method. A plot of the response ratio data versus the amount of analyte added yields a line such that the extrapolated negative x-intercept is the concentration of the analyte in the native sample. In a final approach, three measurements can be made: that of the blank, the blank spiked at one or more analyte concentration, and the blank diluted by some factor. The use of a derived formula relates the relative responses, and spiked addition and dilution amounts, to the concentration of analyte in the native sample. In this presentation, the method of standard addition/standard dilution as an ideal approach to quantitation of drugs in complex and unusual matrix types will be demonstrated from actual forensic case data. Advantages and disadvantages of the technique will also be discussed.

Keywords: Standard addition; Matrix; Quantitation

COMPARISON OF CHEMICAL DERIVATIVES FOR SYSTEMATIC TOXICOLOGICAL ANALYSIS OF AUTOPSY BLOOD USING GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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Aims: Different derivatisation techniques have been developed for drug analysis in whole blood using GC-MS. The method was validated for six drugs of abuse and their metabolites.

Method: A mixed standard stock solution was prepared to give a final concentration of 1µg/ml for each analyte and this was used to spike whole blood. Solid phase extraction (SPE) was carried out using the polymeric phase Phenomenex Strat-X (60 mg/3ml cartridges containing 33µm particles). Blank blood (1ml) was mixed with internal standard solution and different concentrations of mixed standard stock solution, and phosphate buffer (pH 6, 3.5ml). This was centrifuged for 10 minutes and transferred to SPE columns. The extracts, in methanol, were evaporated to dryness and derivatised using three different methods: (a) acylation-esterification with PFPA/ PFP-OH (2:1 v/v, 150 µl); (b) acylation–methylation with PFPA/ trimethylsilyldiazomethane (TMS) (5:1 v/v, 120 µl); (c) silylation with MTBSTFA containing 1% TBDMSCl (30 µl). Reaction vials were heated with a microwave oven for one minute. After derivatisation, vials for methods (a) and (b) were cooled to room temperature and evaporated to dryness under a stream of nitrogen. The derivatised extracts were reconstituted in 50µl of ethyl acetate prior to analysis by GC-MS. Extracts derivatised by method (c) were analysed directly. A Thermo-Finnigan Trace GC-MS instrument was used in selected ion monitoring (SIM) mode except method (c) which used full scan mode. The GC was equipped with an HP5 column (30m x 0.32 mm x 0.25µm) from J&W scientific and split/splitless injection port at 280°C. The oven temperature was at 100°C for 2 min, programmed at 12°C/min to 300°C. Ions monitored for quantitative analysis were as follows:

Derivative	Amp	MA	MOR	BZE	THC	THC-COOH
PFPA/PFP-OH	118	204	414	82	417	459
PFPA/TMS	118	204	414	82	417	489
MTBSTFA	158	172	341	282	371	515

The method was subsequently applied to 35 forensic autopsy case samples.

Results: Recoveries for all drugs of interest were found to be over 70%. Limits of detection (LOD) were calculated as 3 times the standard error of the regression line plus the intercept. LOD's in blood ranged from 0.4 ng/ml to 3.7 ng/ml with PFPA/PFP-OH, 0.3ng/ml to 1.4ng/ml with PFPA/TMS and with MTBSTFA were from 1.9 ng/ml to 7.3 ng/ml. Limits of quantification (LOQ) were calculated as 10 times the standard error. They were ≤12.4 ng /ml with PFPA/PFP-OH, ≤4.7 ng/ml with PFPA/TMS and ≤19.8 ng/ml with MTBSTFA of blood. The case samples analysed were found to contain various drugs of abuse and prescription drugs. The different derivatisation techniques gave varying results; however acylation-methylation gave the best sensitivity and chromatography.

Conclusion: A validated, sensitive and specific method for the extraction and quantification of drugs abuse in blood is presented. An alternative derivatisation method (acylation–methylation) is proposed which gives better sensitivity and improved chromatography for detection and quantification of drugs of abuse compared to silylation or acylation alone.

Keywords: Drugs of abuse, Derivatisation, GC-MS

PREVALENCE OF GHB IN SUSPECTED DUI CASES IN MIAMI-DADE COUNTY, FLORIDA

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According to numerous police and poison control center reports, GHB is a commonly abused substance in Miami-Dade County. Therefore, we wanted to study the prevalence of GHB in urine and blood samples submitted from suspected Driving Under the Influence (DUI) cases to the University of Miami, Forensic Toxicology Laboratory.

Ninety-one consecutive urine samples received from September-December 2003 (whether positive or negative for other drugs) were tested for GHB. In addition, a further 114 urine samples, and 74 blood samples which tested negative for alcohol were tested for GHB and other drugs. Urine samples were received and stored at 4°C in containers without preservatives; blood samples were received and stored at 4°C in gray top vacutainer tubes with sodium fluoride as a preservative.

A method taken from the Journal of Chromatography B, 792 (2003) 83-87 (*Marion Villain, Vincent Cirimele, Bertrand Ludes, Pascal Kintz**) was modified utilizing rapid protein precipitation and liquid-liquid extraction using 40 microL of sample, 40 microL of deuterated internal standard, 90 microL of cold acetonitrile and high speed centrifugation. The supernatant was then carefully evaporated to dryness and the residue reconstituted with 70 microL of BSTFA + 1% TMCS and heating at 70°C for 25 minutes to derivatize. Samples were analyzed by GC/MS with a Hewlett Packard Series 5890 GC coupled to an HP Series MSD run in SIM mode. A 5-point calibration curve ranging from 10 mg/L to 200 mg/L was used to determine the amount of GHB present in the specimen.

Of the total 205 urine specimens tested, 5 tested positive for GHB (2.4% positive) with a concentration range of 237 mg/L – 2147 mg/L (mean = 789 mg/L, median = 484 mg/L). The above 205 samples were made up of 91 consecutive urines of which only 1 was positive for GHB (1.1% positive), and 114 samples selected by case history, of which 4 were positive for GHB (3.5% positive). 2 blood samples from the 74 tested were positive for GHB (2.7% positive) with concentrations of 147 mg/L and 235 mg/L. A summary of the positive findings can be seen in the Table.

Our findings would suggest that either GHB is not as prevalent as it is thought, or that users are so impaired that they don't attempt to drive a vehicle until metabolism and/or excretion have resulted in below detection limit samples, which may account for our low positivity rates. Of interest to note is the concurrent use of GHB and Methamphetamine.

Case one Urine	**GHB:** 2147 mg/L **Other drugs present:** Ibuprofen
Case two Urine	**GHB:** 609 mg/L **Other drugs present:** Amphetamine, Methamphetamine, Naproxen
Case three Urine	**GHB:** 469 mg/L **Other drugs present:** Amphetamine, Methamphetamine
Case four Urine	**GHB:** 237 mg/L **Other drugs present:** Amphetamine, Methamphetamine, MDMA, PPA, Pseudoephedrine, Dextromethorphan, Ecgonine methyl ester, Oxazepam, Alprazolam, Hydroxy-Alprazolam
Case five Urine (These belong to the same defendant) **Case five Blood**	**GHB:** 484 mg/L **Other drugs present:** Amphetamine, Methamphetamine **GHB:** 147 mg/L **Other drugs present:** Acetone 0.003 g/100mL, presumptive positive Amp. class
Case six Blood	**GHB:** 235 mg/L **Other drugs present:** Methamphetamine

Keywords: GHB, GC/MS, DUI

SEPARATION OF AMPHETATIME AND PIPERAZINE DESIGNER DRUGS BY CAPILLARY ELECTROPHORESIS

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The recent emergence of a new class of piperazine-type compounds has brought about the need for laboratory screening methods for both seized drugs and toxicological samples. These piperazine compounds, which include 1-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP), exhibit comparable physiological effects and can be substituted for the classic amphetamine-type drugs. We have optimized a chiral capillary electrophoresis (CE) separation that detects a set of 6 piperazine and 4 chiral amphetamine compounds in less than 23 minutes using a 200 mM phosphate buffer at pH 2.8 with 20 mM hydroxypropyl-β-cyclodextrin (HPβCD). A liquid-liquid back extraction and a solid phase extraction (SPE) were developed for urine samples. The SPE used an altered version of the Varian Bond Elut Certify Amphetamines in Urine procedure. In addition to the above standard compounds, a series of "clandestine" BZP diHCl samples were also analyzed to assess the ruggedness of the method. One of the Internet synthetic methods yielded 1,4-dibenzylpiperazine, an unscheduled analog of 1-BZP. All peak ratios were verified by LC-MS. The novel CE separation was tailored to simultaneously detect piperzine compounds in addition to amphetamine-like drugs. Since the reported effects of these two classes of drugs are strikingly similar, this method will greatly benefit laboratory analysis where the abused or seized substance is in question. Distinct migration time and UV-spectral data were obtained for all compounds of interest.

Keywords: Piperazine, Amphetamine, Capillary electrophoresis

IDENTIFICATION AND ISOLATION OF FILAMENTOUS FUNGI IN SAMPLES OF *CANNABIS SATIVA L.*

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Cannabis sativa L. (Marijuana) is considered an illegal drug being widely used mostly by young adults. The plant dried after harvesting and stored under favorable environment to grow fungi which is spread in nature. Since literature lacks studies related to filamentous fungi present in *Cannabis sativa L...* The objective of this study was to isolate and identify filamentous fungi in samples of *Cannabis sativa L...* retained in Campinas area and analyzed in The Laboratory of Forensic Toxicology. Sixty samples were used divided as follows: 30 samples with evident contamination and 30 without any traces of contamination. First, 5 grams of sample were macerated in 50 ml of sterile distillated water to isolate the fungi. Next, samples were filtered and placed in sterile tubes. Volume transferred to sterile Petri dish was 1ml with 15 ml of Sabouraud Dextrose Agar, subsequent homogenizing and after solidification it was incubated in room temperature for five days. If colony growth was found identification was carried out with macro and microscopy analysis. Most common species of fungi found were as follows: *Aspergillus, Fusarium, Penicillium and Scopulariopsis*. For this reason, *Cannabis sativa L.* represents a source of fungi contamination as fungi were found even in samples that were not visibly contaminated. These mycotoxigenic fungi might cause allergy in hypersensitive individuals, diseases in immune suppressed patients, or intoxication through ingestion of contaminated samples, therefore presenting a potential risk for users and toxicologists as well.

Keywords: Fungi, *Cannabis sativa*, Contamination.

LC-MS/MS IDENTIFICATION OF ETHYL SULFATE - ANOTHER DIRECT MARKER FOR RECENT ETHANOL INTAKE

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Besides ethyl glucuronide (EtG), fatty acid ethyl esters (FAEE) and others, one more direct ethanol-metabolite – ethyl sulfate (EtS) - has been detected by LC-MS/MS in human urine samples. The structural characterization has been performed by LC-MS/MS using electrospray ionization with precursor-ion scan and product-ion scan techniques. After the synthesis of a deuterated analogue, the urinary excretion profile has been investigated in samples obtained from volunteers up to 44 hours after drinking known amounts of alcohol (9 and 18 g, respectively). Ethyl sulfate was detectable up to 24 hours in urine, i.e., 16 to 22 hours longer than ethanol could be detected with a headspace GC/FID method in urine. In summary, the urinary excretion profile of EtS was similar to that of ethyl glucuronide, which is characterized by longer detectability than ethanol in urine samples – and therefore is a marker for ethanol consumption covering the time range of up to approximately three days after severe ethanol abuse. The results of structural characterization, way of synthesis of the deuterated analogue, method validation data including analyte stability in urine and stock solution are presented. EtS can be used for proof of ethanol intake besides EtG. The presented method fulfills forensic-toxicological guidelines for compound identification by chromatographic separation, use of deuterated internal standard, quantitation and identification with precursor and two product ions being monitored as target and qualifier ions.

Key words: Ethyl sulfate, Direct ethanol marker, LC-MS/MS

DETERMINING THE USE OF N^2-ETHYL GUANINE AS A BIOMARKER FOR FATAL ALCOHOL SYNDROME

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Background: Fetal alcohol syndrome (FAS) and fetal alcohol spectrum disorders (FASD) represent the largest categories of *preventable* mental retardation syndromes and birth defects. It is theorized that ethanol's toxicity is in part mediated by damage to DNA. In DNA, ethanol's primary metabolite, acetaldehyde forms an N^2-ethylguanine DNA adduct. The duration of this adduct, its affect on DNA replication, and its repair mechanism are currently unknown. A case study is presented to assess N^2-ethylguanine as a potential alcohol biomarker with possible clinical utility in predicting the risk of FAS/FASD in infants born to alcohol-using women.

Methods: The case subject was a 20 year old who reported binge drinking on hard alcohol for the first 5 weeks of her pregnancy. The separation and quantification of N^2-ethylguanine from unadducted nucleosides was performed by an in-house protocol. DNA was purified from whole blood using a commercial genomic DNA purification kit. The isolated DNA was completely hydrolyzed into monomer bases using established digestion procedures to liberate purine bases; recovery of the purine bases was >96%. The N^2-ethylguanine DNA adduct was isolated and quantified by reversed phase HPLC with isocratic elution and UV/fluorescence detection. Bases were identified by retention time and by standard spiking. Base quantitation was by calibration curves of standard peak height signals.

Results: The identification of N^2-ethylguanine by HPLC was confirmed through standard spiking and LC/MS. HPLC precision for the N^2-ethylguanine was 0.2% for peak area or 3.9% for peak height. Standard curve linearity was to 15 µmol ($r = 0.9999$). Preliminary analytical sensitivity was 29 nmol (HPLC/UV detection), 2.9 nmol (HPLC/fluorescence detection), and 20 fmol (LC/MS). No interference was found from free bases. The subject's N^2-ethylguanine concentration determined by LC/MS at 20 weeks gestation was 11.7 pmol/mg DNA, which declined to 8.3 pmol/mg DNA by 30 weeks. Her adduct levels are higher than a comparison nonpregnant social drinker whose N^2-ethylguanine concentration was 6.9 pmol/mg. Reproducibility of these initial subject results is pending. The subject's fullterm daughter was in the 75th percentile for weight, 17th percentile for length and 10th percentile for head circumference, an indicator of FAS.

Conclusions: Initial studies indicate the acceptability of this method for the separation and detection of purines, including the N^2-ethylguanine. Additional subject data is needed to confirm the adduct's clinical utility.

Public Health Implications: Clinical validation of N^2-ethylguanine as a biomarker for alcohol damage will fortify the established panel of alcohol biomarkers, aiding in the identification of pregnancies at risk for FAS/FASD and elucidation of the mechanisms of alcohol pathology.

Keywords: DNA, Biomarker, FAS

LC/MS/MS DETERMINATION OF LSD, ISO-LSD, AND THE MAIN METABOLITE 2-OXO-3-HYDROXY-LSD IN WHOLE BLOOD AND URINE

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Aim: To develop simple LC/MS/MS applications that identify, confirm and quantify rare potent drugs in forensic cases. An application with the very potent hallucinogenic drug LSD is shown.

Method: A liquid chromatography mass spectrometric (LC/MS/MS) method has been developed for the determination of LSD in whole blood and urine. Furthermore, determination of the common LSD impurity iso-LSD (inactive) and the main metabolite 2-oxo-3-hydroxy-LSD were included. The procedure involved a simple liquid-liquid extraction of 1 g sample containing the analyte and LSD-D_3 (internal standard) with butyl acetate at pH 9.8. After centrifugation, the organic fraction was removed and evaporated to dryness at 40°C and reconstituted in 100 µl mobile phase. A gradient LC system (HP 1100 system, Agilent tech.) with a Zorbax SB C18 (30 x 2.1mm, 3.5 µm) was used to separate the analytes within 10 min. Identification, confirmation and quantification were done by positive electrospray ionisation with a triple quadrupole mass spectrometer (Quattro micro, Waters) operating in multiple reaction monitoring (MRM) mode. For each analyte two MRM's were set up, one for quantification and one as qualifier using one precursor ion and two product ions per analyte. The ratio between the responses of the two MRM's was used for identification purposes along with the retention time.

Results: The curves of extracted whole blood standards were linear over a working range of 0.01 to 5.0 µg/kg for LSD and iso-LSD of both transitions. The limit of quantification (LOQ) of LSD and iso-LSD was 0.01 µg/kg in whole blood, while LOQ of 2-oxo-3-hydroxy-LSD was 0.5 µg/kg. The repeatability expressed by relative standard deviation (RSD) was better than 10% and the relative accuracy was between 92-99% for LSD and 89-108% for iso-LSD, respectively. The ratio was determined for each analyte and a RSD of max. 10% was confirmed.

The method was applied for a case investigation involving a 26-year-old man who admitted to have been abusing LSD. Blood concentrations of LSD and iso-LSD were determined to 0.27 µg/kg and 0.44 µg/kg. 2-oxo-3-hydroxy-LSD was detected in the urine sample and confirmed the abuse of LSD. The case illustrated the importance of analyte separation before MRM detection of a sample. The chromatographic separation of LSD and iso-LSD were of importance because the diastereoisomers have identical fragmentation paths leading to fragments of equal m/z ratio.

Conclusion: The method was proved to be suitable for forensic cases being simple, reproducible and selective. However, it was observed that monitoring typical fragments of LSD without chromatographic separation is inadequate. After the development of this LC/MS/MS method, the initial screening by RIA (radioimmunoassay) for LSD was cancelled because it was too time-consuming and expensive considering the low number of investigations/cases per year.

Keywords: LSD, Whole blood, LC/MS/MS

LC-MS/MS METHOD DEVELOPMENT FOR A PACLITAXEL ASSAY

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The development of a method with liquid chromatography coupled to mass spectrometry (LC-MS) for the determination of the anticancer drug paclitaxel in plasma is described. The goal of the method is to produce accurate and reproducible data, in a sensitive assay. Therefore, factors that influence reproducibility are investigated. The method consisted of a basic liquid/liquid extraction of plasma with methyl-*t*-butyl ether. Subsequently, samples were analyzed using a standard narrow bore reversed phase column. MRM transitions of paclitaxel and the internal standard (cephalomannine) were monitored. Special attention was given to two typical electrospray phenomena: adduct formation and matrix effect. First of all, adduct formation was investigated. In the absence of additives like formic acid or ammonium acetate in the mobile phase, reproducibility of the LC-MS method was decreased, when samples of varying alkali metal content were analyzed. Already at a low concentration of additive in the mobile phase reproducibility increased to an acceptable level. Therefore, a small amount of additive was incorporated in the mobile phase. Different mobile phase additives were tested for optimum sensitivity and reproducibility. Secondly, matrix effect was examined, especially the influence of the vehicle used in the commercial formulation of paclitaxel (Taxol): Cremophor EL. Ion suppression by formulation vehicles has been reported for e.g. polyethyleneglycol 400. In view of the application of this method in a pharmacokinetic study with Taxol, investigation of the effect of Cremophor EL on the ionization was incorporated in the method development. When an isocratic LC run was used, ion suppression was noticed mainly as a result of carry-over to subsequent runs. Changing the mobile phase to a higher percentage of organic phase and incorporating a gradient step (column wash) resolved the matter. The strategy to evaluate and eliminate adduct formation as well as matrix effects can also be applied to LC-MS determinations of other compounds and in fact should become an essential part of a validation protocol.

Keywords: Paclitaxel, Method development, LC-MS

QUANTITATION OF PHENAZEPAM IN BLOOD BY GC-MS IN POSITIVE DRUG-DRIVING CASES IN FINLAND

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Phenazepam (7-bromo-5-(ortho-chlorophenyl)-2,3-dihydro-*1H*-1,4-benzodiazepin-2-one) is white or white with cream-coloured crystal powder that is insoluble in water and slightly soluble in ethanol. After a single oral dose of 2 mg, the peak plasma concentrations of 8-15 ng/ml can be attained in 3-4 hours. Effective therapeutic concentration for persons with neurosis is in the range of 30-70 ng/ml. Phenazepam is comparable with lorazepam in respect of the dose and therapeutic action.

Phenazepam is not medically used in Finland. However it is misused. Therefore, a reliable and rapid method for phenazepam analysis is necessary in Finland. In the present method the phenazepam was analyzed together with diazepam, midazolam, alprazolam, nordiazepam, oxazepam, temazepam, chlordiazepoxide, nitrazepam, bromazepam and lorazepam and their metabolites.

The GC-MS procedure contained liquid-liquid extraction and derivatization by *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA). Analysis was performed in selected ion monitoring (SIM) mode. The developed method was validated and the reliability of the developed method was tested on the basis of linearity, accuracy and precision. Phenazepam has been found among drivers suspected for driving under influence. Year 2003 there were 20 positive phenazepam cases and the concentrations found from Finnish drivers varied from 18 ng/ml to 400 ng/ml.

Keywords: Phenazepam, GC-MS, Quantitation

PHENMETRAZINE OR EPHEDRINE? FOOLED BY LIBRARY SEARCH

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Phenmetrazine, a central nervous system stimulant, currently abused as an anorectic agent, was positively identified by library search after injecting a methanolic extract of an ephedrine containing powder using different GC-MS configurations. Phenmetrazine is a controlled substance as described in the UN Convention of Psychotropic Substances 1971, while ephedrine is used in over-the-counter medications as a vasoconstrictor. However, very recently, the Food and Drug Administration issued a final rule prohibiting the sale of dietary supplements containing ephedrine alkaloids because such supplements present a health risk.

GC-MS analysis of a methanolic extract of the herbal product containing ephedrine resulted in a peak with a nearly identical retention time as ephedrine but identified as phenmetrazine after a library search. It is known that formaldehyde contamination in solvents such as methanol can result in conversion of ephedrine-like compounds. Ephedrine reacts with formaldehyde to form 3, 4-dimethyl-5-phenyl-1, 3-oxazolidine with a similar molecular weight as phenmetrazine (1). The fragmentation pattern is also similar because both chemical structures differ only in the position of one carbon unit. Thus, library searches alone can lead to erroneous conclusions. Experiments using a HP 7683 cold on-column- HP 6890GC-HP 5973 MSD configuration and a split injector in combination with a 3400 Varian GC-Finnigan Magnum ion-trap MSD demonstrated that high injection temperatures influence the conversion speed. This was evidenced by injection of a freshly made ephedrine standard in methanol using injector temperatures from 60 up to 300°C.

Attention should be paid to the possibility of chemical and/or thermal conversion of analytes in a sample and/or in the GC-injector, as this can lead to interpretation difficulties and erroneous identification of analytes in a toxicological screening. As demonstrated by this case, ephedrine-containing samples are of particular interest in view of the judicial implications.

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Keywords: GC-MS, Ephedrine, Conversion.

AN IDA MEDIATED LC-MS/MS SCREENING PROCEDURE WITH SEMI-QUANTITATIVE POTENTIAL

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Systematic toxicological analysis (STA) in forensic toxicology comprises general unknown screening (GUS) procedures, whether or not restricted to well-defined subgroups, as well as specific confirmation and quantitation of individual compounds. Even though in some forensic cases, the substance involved is known or strongly suspected, the possibility that other toxic compounds may have contributed to the observed biological effect cannot be excluded. Efficient and extensive screening strategies are therefore indispensable. This study evaluated an IDA-mediated LC-MS/MS screening methodology, qualitatively as well as quantitatively, and shows its scope and limitations in a forensic setting. After all, an LC-MS screening strategy could on the one hand reduce the off-line sample preparation mandatory in GC-MS, because relatively non-volatile compounds can be analyzed as such, and on the other hand increase the range of compounds amenable to MS. IDA is an artificial intelligence based product-ion scan mode providing automatic "on-the-fly" MS to MS/MS switching. This laboratory introduced in a preliminary communication, as first, the concept of IDA-based LC-MS/MS screening using a Q-TOF[1]. By performing information dependent scanning at two different fragmentation energies, two collision-induced dissociation (CID) spectra for each of the detected compounds are generated. As the MS/MS spectra derive from a single precursor ion, a very selective and very specific method was devised. Additionally, advance knowledge about the xenobiotics that could be responsible for a certain intoxication is not necessary. Limitation of the MS/MS acquisition time to an acceptable minimum resulted in an almost instant switch back to the MS mode. As such, this approach provided MS chromatograms that still could be of use for semi-quantitative purposes. Since former studies revealed that the IDA intensity threshold, unequivocally related to the background noise, seemed to be a critical parameter, the solid phase extraction procedure, the liquid chromatographic conditions and the mass spectrometric parameters all were optimized in advantage to IDA. Optimization of the SPE procedure was performed by means of experimental design. Whole blood was preferred as biological matrix because of its relevant toxicological characteristics, although this did not facilitate the analysis since the drug concentration in whole blood are relatively low compared to urine samples. The influence of two different threshold values, i.e. 100eV and 400eV, on the qualitative and quantitative results was examined. Finally, the screening procedure we developed was benchmarked on the one hand qualitatively against the results obtained from traditional GUS approaches in a number of routine toxicological laboratories (37 samples). To that end, immunochemical techniques (EMIT, RIA), HPLC-DAD, GC-MS and GC-NPD techniques were applied to screen the whole blood samples. On the other hand the IDA-mediated screening strategy was compared semi-quantitatively against established LC-MS/MS methods (7 samples). From a qualitative point of view the procedure performed exceptionally well: when applying a threshold of 100eV, mostly all of the drugs detected by the conventional techniques were identified, as well as additional drugs that were not previously reported. The procedure proved well-suited for an initial semi-quantitative assessment, as is customary in e.g. forensic toxicology before accurate intoxication levels are determined using targeted analytical analysis.

[1] Decaestecker et al. Rapid Commun. Mass Spectrom. 2000, 14, 1787-1792.

Keywords: LC-MS/MS, STA, IDA

DETERMINATION OF NALOXONE AND NORNALOXONE IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - ELECTROSPRAY IONIZATION - TANDEM MASS SPECTROMETRY

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Naloxone is a μ-opioid antagonist that has been used clinically for over forty years mainly for the treatment of opioid overdose and to reverse narcotic-induced depression following surgery. A more recent application is the use of naloxone in a combination tablet containing buprenorphine at a fixed ratio of 4:1 (buprenorphine: naloxone) for the treatment of opioid dependence. The purpose of the addition of naloxone to buprenorphine tablet is to prevent diversion of buprenorphine for illicit i.v. use. Because of the low doses of naloxone used in maintenance therapy, sensitive analytical methods are required. This has traditionally proven difficult for 6-keto nor-opiates such as nornaloxone. A highly sensitive method was developed to measure naloxone and its metabolite nornaloxone in human plasma to gain further knowledge about their metabolism and pharmacokinetics. d_3-Naltrexone and d_3-oxymorphone were used as internal standards for naloxone and nornaloxone. Preliminary experiments demonstrated that solid-phase extraction improved the recovery of nornaloxone to 30% compared to 10% using liquid-liquid extraction. Solid-phase extraction was applied for sample preparation using C18 extraction columns and 0.01 M ammonium carbonate buffer (pH = 9). High performance liquid chromatography interfaced by electrospray ionization to a tandem mass spectrometric detector (HPLC-ESI-MS/MS) was used for quantitation. A XTerra MS C18 3.5 μm 2.1x 50 mm column (Waters Corporation, Milford, MA) was used for separation. The mass spectrometer was a Finnigan model TSQ7000 Thermo Quest triple-stage quadrupole. Quadrupole 1 was set to pass only ions at m/z 328, 288, 345 and 305 that correspond to the MH^+ ions of naloxone, nornaloxone and their internal standard d_3-naltrexone and d_3-oxymorphone. The MH^+ ions were caused to undergo collision induced dissociation in quadrupole 2 that produced product ions at m/z 310, 270, 327 and 287 respectively, which were then monitored selectively by quadrupole 3. The calibration range was from 0.075 to 20 ng/mL for naloxone and 1 to 20 ng/ml for nornaloxone with the calibration curve constructed as quadratic with 1/X weighting. The lower limit of quantitation (LLOQ) was determined at 0.075 ng/ml for naloxone and 1 ng/ml for nornaloxone. Specificity for naloxone and nornaloxone was determined from analysis of blank plasma fortified with internal standard only (3 replicates) and with LLOQ concentration (1 replicate) in six different lots of plasma. The primary evaluation was to compare the mean peak area ratio of any signal at the retention time of naloxone and nornaloxone to its internal standard for each lot with the mean peak area ratio of the six LLOQ samples. Mean ratios relative to mean LLOQ ranged from 2.68 to 7.72 with a mean of 5.35% for naloxone and from 8.03 to 25.5 with a mean of 16.5% for nornaloxone. Intra-run accuracy of the LLOQ was within 5.1% of target with intra-run precision within 13.5% for naloxone and within 12% of target and with intra-ran precision within 7.4% for nornaloxone. (Supported by NIDA grant R01DA10100)

Keywords: Naloxone, Nornaloxone, HPLC-ESI-MS/MS

SIMULTANEOUS DETERMINATION OF BUPRENORPHINE, NORBUPRENORPHINE, BUPRENORPHINE-3-GLUCURONIDE AND NORBUPRENORPHINE-3-GLUCURONIDE IN HUMAN PLASMA AND URINE BY LIQUID CHROMATOGRAPHY – ELECTROSPRAY IONIZATION - TANDEM MASS SPECTROMETRY: APPLICATION TO HUMAN PHARMACOKINETICS

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Our previously described method for buprenorphine (BUP), norbuprenorphine (NBUP), buprenorphine-3-glucuronide (BUPG) and norbuprenorphine-3-glucuronide (NBUPG) (SOFT 2003) has undergone a slight modification prior to full validation and use in a pharmacokinetics study. The monitored ions for NBUP and its internal standard d_3-NBUP were changed from 101 (product ion) to 414 (parent ion) and 101 to 417, respectively. This provided stronger signals and a resultant lower limit of quantitation (LLOQ) of 0.1 ng/mL for NBUP.

A full validation using human plasma with a cross-validation to human urine was conducted. The LLOQ was 0.1 ng/mL for all analytes in human plasma. For the urine samples, the LLOQ was 0.5 ng/mL for BUP, BUPG and NBUPG and 2.5 ng/mL for NBUP due to interference in some urine samples. The intra-assay precision, as coefficient of variation (%CV), at 0.1, 0.25, 25 and 40 ng/mL in plasma did not exceed 17.5% at the LLOQ or 12.5% at all other concentrations for all analytes; in urine, the %CV did not exceed 17.4% at the LLOQ or 13.3% at all other concentrations for all analytes. The intra-assay accuracy, as % deviation from target, in plasma did not exceed 12.3% at all concentrations for the four compounds; in urine the % deviation did not exceed 14% at all concentrations. The interassay precision and accuracy was determined at 0.25, 25 and 40 ng/mL; %CV did not exceed 7.0% and the % deviation did not exceed 5.6% for the four analytes. The method has now been used to determine the pharmacokinetics of the four analytes in plasma and urine collected from five patients who had been maintained on a daily sublingual does of 16 mg buprenorphine for at least 21 days. Plasma was prepared from blood collected for 13 time points from just prior to 24 hours after the daily dose and urine collected for the same 24-hour period. The results were as follows:

Pharmacokinetic parameter	BUP	NBUP	BUPG	NBUPG
Plasma				
Cmax $(ng)(mL)^{-1}$	4.47 ± 0.54	6.36 ± 1.84	7.19 ± 3.27	21.5 ± 4.2
Tmax (h)	0.85 ± 0.42	1.00 ± 0.47	0.80 ± 0.41	1.80 ± 0.27
AUC $(ng)(mL)^{-1}(h)$	32.1 ± 3.8	87.6 ± 24.6	25.5 ± 7.9	316 ± 172
24-hour Urine				
Concentration $(ng)(mL)^{-1}$	0.94 ± 0.67	112 ± 58	94.9 ± 27.0	463 ± 153
Amount (% daily dose)	0.014 ± 0.011	1.89 ± 1.10	1.01 ± 0.42	5.43 ± 2.46
Renal Clearance $(mL)(min)^{-1}$	1.09 ± 0.83	48.7 ± 23.9	148 ± 43	59.1 ± 6.8

The current method has been shown to be accurate and precise with sufficient sensitivity to monitor all four analytes in human plasma and urine samples. The pharmacokinetic data, for the first time, show the magnitude of involvement of NBUPG in the clearance of buprenorphine.

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Key words: Buprenorphine, Glucuronide metabolites, Pharmacokinetics

Scientific Session Abstracts:

Behavioral Toxicology

COMPARISON OF BLOOD AND BRAIN CANNABINOIDS CONCENTRATIONS IN 11 HUMAN CASES. CONSEQUENCES ON TRAFFIC SAFETY.

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It is known that cannabis effects on cognitive and psychomotor tasks are proportional to the delta 9-tetrahydrocannabinol (THC) amount consumed but that such impairments are not directly related to THC concentrations in blood. Some studies have also indicated that impairing effects could still be present while THC blood concentrations had dropped to a few ng/ml, but this assessment has never been confirmed by biological data. However, in a number of circumstances such as traffic safety or homicides, forensic toxicologists have frequently been asked to answer this question: according to the blood cannabinoids concentrations, was the subject under influence of cannabis? In order to document this important question, we compared cannabinoids concentrations in blood and brain human samples.

Blood and corresponding brain corresponding samples were provided from legal autopsies performed to determine the cause of death. In 11 cases, cannabinoids were found in blood: these 11 cases were consequently included in this study. All samples were stored at –20°C until analysis. THC, 11-hydroxy-THC (11-OH-THC) and 11 nor-9-carboxy-THC (THC-COOH) were investigated by a gas chromatography – mass spectrometric procedure derived from the method of Kemp et al. (1). Brain samples were initially grinded in pH 7.4 Tris buffer. In 10 cases, the precise location of brain sampling was not known. The results, expressed in ng/g, are indicated in the following table :

Case number	1	2	3	4	5	6	7	8	9	10
THC Blood	0.5	0.6	1.8	2.3	3.0	4.4	11.5	< 0.2	<0.2	<0.2
THC-COOH Blood	2.8	13.0	3.6	6.0	28.7	11.0	66.0	8.9	1.8	6.1
THC Brain	0.9	1.1	2.5	2.9	12.4	19.4	20.8	1.6	2.2	29.9

In one other case, the location of brain sampling was defined. The following table indicates the means (n=3) of the results obtained in locus niger (LN), hippocampus (HIP), occipital lobe (OL), striatum-putamen-pallidum (SPP),
frontal lobe (FL), spinal cord (SC) and corpus callosum (CC).

Case n° 11	blood	LN	HIP	OL	SPP	FL	SC	CC
THC	5.4	35.6	17.9	16.6	20.0	12.6	27.0	38.6
11-OH-THC	2.8	16.6	11.1	11.7	14.7	12.5	12.0	15.8
THCCOOH	38.3	28.8	17.9	27.8	26.1	12.5	28.7	16.8

These results indicate that THC, 11-OH-THC and also THC-COOH may be found in cerebral structures, at high concentrations. The presence of THC-COOH in brain, which had never been reported previously, may raise some new questions concerning THC metabolism. In 3 cases, high THC levels in brain were observed whereas THC was not detectable (<0.2 ng/g) in blood. These results explain the prolonged effects of cannabis and could confirm the hypothesis of the occurrence of possible flash-backs related to a sudden release of THC from lipidic tissues.

As a conclusion, in the field of traffic safety or in other forensic cases, the presence of THC in blood even if concentrations are very low suggests that THC was present in brain simultaneously, and consequently able to impair motor and cognitive functions.

Reference:
1. Kemp MP, Abukhalaf IK, Manno JE, Manno BR, Alford DD, Abusada GA. *J. Anal. Toxicol.* 1995; 19: 285-291.

Key words: cannabis, brain, traffic safety

PROSPECTIVE STUDY ABOUT THE EFFECT OF CANNABIDIOL (CBD) ON THE PHARMACOKINETICS OF Δ^9-TETRAHYDROCANNABINOL (THC) AFTER ORAL APPLICATION OF 10 MG THC AND 5.4 MG CBD IN CANNABIS EXTRACT

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Besides the psychoactive delta 9-tetrahydrocannabinol (THC), varying amounts of cannabidiol (CBD) are contained in hashish and marihuana. Furthermore, cannabis-based medicine extracts and clinical grade cannabis contain high quantities of CBD, which frequently equal the percentage of THC. CBD is known from literature to modify the effects of THC and to have anti-anxiety effects and anti-psychotic benefits and to inhibit the P450 mediated conversion of THC to 11-OH-THC. Therefore, in the present prospective and double blind study with 24 volunteers the concentration vs. time curves of THC and its main metabolites 11-hydroxy-delta9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH) as well as of CBD were measured after oral administration of 10 mg THC alone or in cannabis extract containing 5.4 mg CBD. Additionally, in 12 volunteers the effect of food on the concentration vs. time curves was tested. The analytical method was based on automatic solid phase extraction with C_{18} columns, derivatization with N,O-bistrimethylsilyltrifluoroacetamide (BSTFA) and electron impact ionization gas chromatography-mass spectrometry (GC-EI/MS) with deuterated standards. The limits of detection were between 0.15 and 0.29 ng/ml. For the statistical evaluation, the course of the concentration vs. time curves and the areas under the curves (AUC) were compared and the metabolites as well as metabolite/drug ratios were included.

The maximum plasma concentrations after intake of the cannabis extract ranged between 1.2 and 10.3 ng/ml (mean 4.05 ng/ml) for THC, 1.8 and12.3 ng/ml (mean 4.9 ng/ml) for 11-OH-THC, 19 and 71 ng/ml (mean 35 ng/ml) for THC-COOH and 0.2 and 2.6 ng/ml (mean 0.95 ng/mg) for CBD. The peak concentrations (mean values) of THC, 11-OH-THC, THC-COOH and CBD were observed 56, 82, 115 and 60 min respectively after intake. As a mean slightly higher AUC for THC and slightly lower AUC for THC-COOH were measured after application of THC + CBD when compared with THC alone but the difference between both kinds of intake was not statistically significant. Therefore, an effect of CBD on the pharmakokinetics of THC at the studied concentrations could not be proved.

The data were also compared with the results obtained by Huestis et al. in smoking experiments [1]. Caused by the strong first-pass metabolism, the concentrations of the metabolites were increased during the first three hours after oral drug intake as compared to smoking. Therefore, the concentration ratio THC-COOH/THC is discussed as a criterion for distinguishing between oral and inhalative cannabis consumption. As a result, the differentiation between both ways of drug use based on this ratio is not possible in a practical case, since the exact time of drug intake before sampling is not known, since THC-COOH may be present in excess from previous consumption, and because of the large variation of the ratios within both groups. However, it is shown that the cannabis influence factor CIF introduced by Daldrup et al. for interpretation of driving impairment by the drug [2] can also be used after oral intake.

[1] M.A. Huestis, J.E. Henningfield, E.J. Cone. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J. Anal. Toxicol. 16:276-282. (1992).

[2] T. Daldrup, I. Meininger. Begutachtung der Fahrtüchtigkeit unter Cannabis im Strafverfahren. In: G. Berghaus, H. P. Krüger (Eds.): Cannabis im Straßenverkehr. Gustav Fischer Verlag, Stuttgart 1998, pp. 181-204.

Keywords: Cannabinoids, Cannabidiol, Oral intake

EFFECTS AND BLOOD CONCENTRATIONS OF THC, 11-OH-THC AND THCCOOH FOLLOWING ORAL ADMINISTRATION OF 20 MG DRONABINOL OR OF A CANNABIS DECOCTION CONTAINING 20 OR 60 MG DELTA9-THC

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Smoking and oral ingestion are the most popular methods of Cannabis recreational use. Oral therapeutic administration, such as with Marinol® (synthetic THC (dronabinol) in sesame oil) was introduced in the US to alleviate anorexia in HIV/AIDS patients and to ease nausea in cancer chemotherapy. THC is also contained in hemp foods. In Switzerland, there is currently a great concern about the danger of driving under the influence of Cannabis. Epidemiological studies have indeed shown that Cannabis alone or with alcohol constitute the most frequent group of drugs detected in drivers involved in car accidents or in driving under the influence of drugs. The main objective of our study was to assess the effects of an acute oral intake of a medium to high dose of THC on driving capability. A hemp crude preparation containing 1.5 % delta9-THC and 4.4 % delta9-THC-A was pre-treated at 140°C for 30 min to obtain a powder with 4.9 % delta9-THC. From this powder containing 20 or 60 mg of delta9-THC, hemp milk decoctions were prepared with a yield of about 80%. A double-blind, ethical committee approved study was carried out with eight 20-30 years-old healthy male volunteers who were occasional Cannabis smokers. In 4 successive sessions, volunteers were administered a placebo, a decoction made with 20 or 60 mg delta9-THC or 20 mg dronabinol capsules in a randomised order. Blood samples were taken before administration and 1, 2.5, 4, 5.5, 7, 10 and 24 hours following ingestion. In the same time-period, clinical examination and psychological tests were performed with a driving simulator. Blood levels of cannabinoids were determined by NCI GC-MS according to a method adapted from Huang et al[3] with limits of quantification of 0.3 ng/mL for THC, 0.8 ng/mL for 11-OH-THC, and 0.1 ng/mL for THCCOOH.

Because of anxiety events, which resolved spontaneously, two volunteers were withdrawn from the study after ingestion of the medium dose. Mean maximal concentrations [ng/mL] for THC, 11-OH-THC and THCCOOH as well as the times [hours] to the maximum blood levels are listed below:

Preparation	Parameter	THC	11-OH-THC	THCCOOH
dronabinol	Tmax	1	4	5.5
20 mg Δ^9-THC	Cmax	2.8	3.9	27.8
milk decoction	Tmax	1	1	4
16.5 mg Δ^9-THC	Cmax	3.8	4.7	27.8
milk decoction	Tmax	1	2.5	2.5
45.7 mg Δ^9-THC	Cmax	8.4	12.8	66.2

The individual results show there was a considerable inter-subject variability. Mean maximum concentrations of 11-OH-THC exceeded the corresponding levels of THC. A 2 to 3 fold increase in cannabinoid concentrations was achieved following ingestion of the strongest decoction. Almost similar levels were measured after intake of the same dose of THC, regardless of the type of preparation (synthetic THC or crude plant extract). Obvious effects were observed with the tracking test showing a 50 % mean highest performance decrease after drinking the milk decoction containing 45.7 mg THC. Although a relatively high dose of THC was taken and significant psychoactive effects and performance reduction were noticed, THC and THCCOOH concentrations were much lower than those which are generally measured after marijuana smoking. In conclusion, this study shows that oral intake of delta9-THC may decrease human performance and exemplify the risks of driving under the influence of Cannabis.

Key words: dronabinol, cannabis, driving under the influence of drugs

[3]Huang W et al. J. Anal. Toxicol. **25:** 531-537, 2001

REASONS FOR OVERESTIMATION OF THE ROLE OF CYP2D6 IN HUMAN METABOLISM OF AMPHETAMINE PRECURSOR DRUGS USING THE DARK AGOUTI RAT MODEL

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Background: Female Dark Agouti rats (fDA) have been proposed as a model of the human CYP2D6 poor metabolizer phenotype (PM) allowing a preliminary screening for CYP2D6 substrates. This rat model has successfully been used by the author's working group to investigate the role of CYP2D6 in human metabolism of the new designer drug TFMPP (1-(3-trifluoromethylphenyl)piperazine). However, the crucial role of CYP2D6 in N-dealkylation of amphetamine precursors (e.g. fenproporex) to amphetamine predicted by this rat model could not be confirmed using recombinant human CYPs and human liver microsomes (HLM). Four different CYP isoforms, namely CYP1A2, CYP2B6, CYP2D6 and CYP3A4, were involved in this metabolic step with CYP2D6 obviously not being of major importance. Using the data on intrinsic clearance for the particular isoforms the percentage of contribution of each isoform to the entire microsomal clearance in vitro was calculated for fenproporex. The highest contribution was found for CYP2B6 (65 % for *R(-)* and 72 % for the *S(+)*-enantiomer) and the lowest values were found for CYP2D6 (7 % and 4 % for *R(-)* and *S(+)*-enantiomers, respectively). The aim of this study was to elucidate the reasons for the failure of the rat model. As a characteristic test reaction for CYP2B6 activity (CYP isoform with the highest contribution), bupropion side chain hydroxylation was chosen.

Methods: Data for calculation of intrinsic clearances for fenproporex N-dealkylation were taken from previous studies with recombinant CYPs. Bupropion hydrochloride (R,S-2-(tert-butylamino)-3'-chloropropiophenone; amfebutamone; BU) was administered to Wistar (model of the human CYP2D6 extensive metabolizer phenotype) and Dark Agouti rats for toxicological diagnostic reasons according to the corresponding German law. Urine was collected separately from the feces and analyzed directly after sampling. After enzymatic cleavage of conjugates and centrifugation, bupropion and hydroxy-bupropion were separated and quantified using an Agilent Technologies (AT, Waldbronn, Germany) AT 1100 series atmospheric pressure chemical ionisation (APCI) electrospray LC-MSD, SL version and an LC-MSD ChemStation using the A.08.03 software. Gradient elution was achieved on a Merck LiChroCART® column (125 x 2 mm I.D.) with Superspher®60 RP Select B as stationary phase and a LiChroCART®10-2 Superspher®60 RP Select B guard column. The mobile phase consisted of ammonium formate (5 mM, adjusted to pH 3 with formic acid) (eluent A) and acetonitrile (eluent B). Human urine samples from volunteers after ingestion of BU were analyzed in the same way.

Results and Discussion: BU and its metabolites could easily be separated using the applied LC-MS conditions. Characteristic fragments in the APCI-mass spectra allowed easy discrimination of the side-chain hydroxy from the ring hydroxy metabolites. Bupropion side chain hydroxylation was chosen as specific test reaction for CYP2B activity. Indeed, the side chain hydroxy bupropion (HO-BU) was the major BU metabolite in human urine samples, whereas it was almost completely absent in urine of the two rat strains. CYP2B6 seems to play a major role in human metabolism of amphetamine precursors. BU side chain hydroxylation, a characteristic test reaction for this isoform, hardly occurs in either rat strain. As a consequence, it could be possible, that for lack of a corresponding CYP2B isoform, CYP2D isoforms take over such metabolic reactions in rats leading to an overestimation of the role of CYP2D for human metabolism. In addition, it should also be kept in mind, that the lower extent of ring hydroxylation of amphetamine precursors under *in vitro* conditions may also contribute to the differences found between animal *in vivo* models and human *in vitro* test systems.

Keywords: Dark Agouti rats; cytochrome P450; amphetamine precursor drugs

CORRELATION OF SALIVA AND BLOOD ESTAZOLAM CONCENTRATIONS WITH BALANCE CHANGES IN SUBJECTS AFTER ADMINISTRATION OF THE DRUG AND/OR ALCOHOL

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In 1992-1999, at the Institute of Forensic Research in Krakow, blood samples taken from 83 drivers involved in road incidents tested positive for morphine. Benzodiazepines (BZD) were found in 60% of the cases. In 2002-2003, the number of drivers under the influence of drugs involved in accidents was 166. There was a high prevalence (65%) of THC and amphetamines detected. 17% of the population were under the influence of morphine and other medications, and 23% tested positive for BZD. In 2002, 278 drivers were questioned about driving a car after intake of a medicinal drug. 134 respondents reported driving a car while taking a medicinal drug; 41% of them used BZD. Although the approach to the presence of morphine, THC, cocaine and amphetamines in the blood of drivers should without doubt be one of "zero-tolerance", the situation concerning the legal limit for BZD is not so clear.

On the basis of the results both of surveys and laboratory examinations, it can be stated that the BZD's most often used in Poland are diazepam and estazolam. Therefore, estazolam was chosen as a model substance for analysis of balance changes in persons influenced by the drug. The balance disturbance that occurs at the legal limit (0.5 g/L) of alcohol in blood was used as a reference point.

Concentrations of estazolam in blood and saliva samples were determined by the LC-MS/APCI method after LLE. Blood and saliva samples were taken from 25 healthy volunteers who received a single oral dose of 1 mg of estazolam. The samples were collected every hour, for four hours. Half an hour before the last sampling, volunteers received a dose of ethanol that led to a blood concentration of about 0.5g/l. On the next day, the same volunteers received alcohol alone at the same dose, and a blood and saliva sample was taken 0.5 h later.

The LC-MS/APCI method had the following validation parameters [ng/mL]: LOD - 0.33 and 0.63, LOQ - 1.09 and 1.05, LOL - up to 200 and 20 for blood and saliva respectively.

The dose of estazolam produced average blood concentrations of 21, 24, 24 and 29 ng/mL, and saliva of 2.8, 1.9, 1.9 and 2.2 ng/mL at the respective sampling points. The correlation coefficient between blood and saliva concentrations was 0.50.

For estimation of balance changes in persons influenced by estazolam, alcohol, and estazolam with alcohol, posturography was used. Using the Langevin equation, the diffusion matrix and the friction coefficient were calculated. For each of the tested persons, 8 stabilograms were obtained, two (with open and closed eyes) for each of the four studied situations: control (before estazolam or alcohol administration); after intake of alcohol alone; 2 h after intake of estazolam alone; as well as after intake of ethanol together with estazolam.

It was shown that the values of the friction coefficient decreased when persons kept their eyes closed, from: 9.45 to 7.25 s^{-1}, 9.45 to 6.95 s^{-1} and 9.83 to 6.77 s^{-1} after administration of estazolam, alcohol, and alcohol with estazolam respectively. These changes showed a trend when under the influence of estazolam ($P=0.078$) and ethanol ($P=0.057$), but were statistically significant for estazolam with ethanol ($P=0.036$) as compared to the placebo condition. The values of the friction coefficient after administration of alcohol and estazolam did not differ statistically ($P=0.677$).

The results show that the friction coefficient can be used as an indicator that allows us to assess if a person is under the influence of a medicine that disturbs his/her balance.

Keywords: Estazolam, Friction Coefficient, Posturography.

EVALUATION OF THE POST-ROTATIONAL NYSTAGMUS TEST (PRN) IN DETERMINING ALCOHOL INTOXICATION

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Aims: With a blood alcohol concentration (BAC) legal limit of 0.5 g/kg (since 1965), Slovenia's road traffic legislation has increasingly oriented the work of forensic toxicologists towards quality assurance of alcoholometric analyses of BAC and breath alcohol concentration (BrAC). The introduction of a Standardised Field Sobriety Test (SFST) for identifying suspected DUI offenders raised our interest to evaluate the post-rotational nystagmus test or Tashen test, a part of the physician's examination for detecting alcohol intoxication. Post-rotational nystagmus is induced by suddenly stopping the rapid rotation of the body; large slow movements of the eyeballs occur in the opposite direction to the direction of rotation.

Performing the test: The person is turned with open eyes in a tight circle 5 times around his axis (while sitting on a swivel chair). Time is measured after the chair stops and the person fixes his gaze on an object (e.g., a pencil or finger), which the physician holds at a distance of approximately 30 cm from his eyes.

Materials and methods: The study included the results of 1,006 PRN tests performed during medical examinations for DUI cases at the Institute for Forensic Medicine in Ljubljana in the years 1998–2002. Cases with a combination of alcohol and drugs were excluded. The evaluation of PRN test results with BAC as a reference was based on classification into the following categories and characteristics: true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and accuracy.

Results: Measured interval values of post-rotational nystagmus time ranged from 1 to 45 s, mean value 14, median 13; BAC values were between 0.0 and 3.16 g/kg, mean 1.12 g/kg, median 1.14 g/kg. The values of post-rotation nystagmus time and BAC show a positive correlation, which is statistically significant (r = 0.54, $p < 0.01$). For the regression line, the following formula was obtained: PRN = 6.0 x BAC + 7.2.

Raising the threshold values for post-rotational nystagmus time (from 6 to 16 s) shows an increase in sensitivity accompanied by a decrease in specificity. The optimal cut-off value of 10 s for post-rotational nystagmus time was chosen with the help of a Receiver Operating Characteristic curve (ROC curve) for BAC limit 0.5 g/kg (TP=584, FP=43, FN=229, TN=150, sensitivity=0.718, specificity=0.777, PPV= 0.931, NPV= 0.396, accuracy=0.730).

Conclusions: According to the area under the ROC curve, the post-rotational nystagmus test is a fair test for predicting alcohol intoxication over 0.5 g/kg. However, as a part of the physician's examination it can contribute to the description of the clinical state.

Key words: post-rotational nystagmus, alcohol intoxication

DETECTION OF COMMONLY ABUSED DRUGS IN URINE OF SEXUAL ASSAULT COMPLAINANTS

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There are approximately 100,000 reported cases of sexual assault in the U.S. every year. It is estimated (Bureau of Justice Statistics) that there are more than 300,000 sexual assaults every year, three times the number actually reported. Recently, the use of "date-rape" drugs to incapacitate someone has received considerable coverage in the media. However, before toxicologists can examine what "date-rape" drugs are present in a sexual assault complainant, it is important to know what drugs of abuse are also commonly found. This project is designed to estimate which drugs of abuse are found in sexual assault complainants through a random sample from four reasonably representative US jurisdictions. Sites include locations in Washington, Texas, California, and Minnesota.

Sexual assault complainants are asked when presenting to the hospital if they would like to take part in this study. If they agree, consent forms are filled out and the complainant provides a urine sample following a protocol approved by the UIC IRB. The sample is then sent to our laboratory for proper handling and storage. At the end of the subject recruitment phase of the study, 31 sexual assault complainants had been recruited at the Texas location, 56 from the California site, 15 from Washington, and 43 in Minnesota, for a total of 145. The racial distribution of the sample is: 70.3% White, 8.3% Black, 13.1% Latino, and 8.3% other/unknown. The ages of the complainants range from 18 to 56 with the highest number in the 21-25 age cohort.

Urine samples collected from all of the complainants are screened by immunoassay for the following drugs of abuse (values in parentheses are corresponding cut-off values): ethanol (40 mg/dL), amphetamines (250 ng/mL), opiates (50 ng/mL), PCP (10 ng/mL), cannabinoids (10 ng/mL), methadone (100 ng/mL), barbiturates (100 ng/mL), and benzodiazepines (100 ng/mL). Because drug-facilitated sexual assault victims may have only been given a single dose, low cut-off values for the EMIT screen were used to achieve maximum sensitivity. All presumptive positive samples are confirmed by GC-MS following extraction and derivatization if appropriate. To date, 125 specimens have been analyzed. Confirmed positives include 13.6% for cocaine, 28% for marijuana, 2.4% for benzodiazepines, 6.4% for opiates, and 7.2% for amphetamines. These preliminary results suggest that complainants of sexual assault have more drugs in their system than the normal population.

In order to determine if sexual assault complainants are more or less likely to abuse drugs, our results will be compared to general population drug use data from NIDA's Monitoring the Future and the National Household Survey on Drug Abuse conducted by SAMHSA. The confirmed positives will also be examined by race, age, and geographic location to determine if any trends are apparent.

Key Words: Sexual assault, drugs of abuse, GC-MS.

TOXICOLOGICAL FINDINGS IN CASES OF ALLEGED DRUG FACILITATED SEXUAL ASSAULT IN THE UNITED KINGDOM

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The Forensic Science Service (FSS) is the major provider of forensic services in the UK. It has several laboratories throughout the country with two providing forensic toxicology services. These two laboratories annually deal with approximately 500 cases of alleged drug facilitated sexual assault (DFSA) submitted from various UK police forces.

This study outlines the results from 1014 cases of claimed drug facilitated sexual assault analysed at the Forensic Science Service, London Laboratory between January 2000 and December 2002. As and where appropriate, either a whole blood sample or a urine sample from the complainant was initially analysed for alcohol and common drugs of abuse. The test for alcohol was by gas chromatography and for common drugs of abuse by immunoassay with positive results being confirmed by gas chromatography-mass spectroscopy (GC-MS). Common drugs of abuse tested for include cannabis, amphetamine, Ecstasy, cocaine, opiate drugs, methadone, barbiturates and the benzodiazepine drugs diazepam and temazepam. All urine samples (or blood sample if urine not collected) were further tested for potentially stupefying drugs and their metabolites by either positive or negative ion GC-MS (as applicable). These tests have been shown to have the low limits of detection required to detect the drugs of concern. Potentially stupefying drugs included a range of other benzodiazepine drugs (including flunitrazepam) and related drugs such as zopiclone, gammahydroxybutyrate (GHB) and a range of chemically basic pharmaceutical drugs such as ketamine and other medicinal drugs with sedative properties. The urine samples were also tested for trichlorinated compounds (e.g. chloral hydrate) by a colour test.

The results are interpreted with respect to the numbers of each drug detected. An attempt has been made to distinguish between voluntary use and involuntary ingestion by using information provided by the investigating police officer (with follow-up discussion where necessary). Furthermore, in those cases where alcohol was detected, the most likely blood alcohol level at the time of the alleged incident has been calculated.

Alcohol (either alone or with an illicit or medicinal drug) was detected in 46% of all cases and in 81% of the cases in which the samples were taken within 12 hours of the alleged incident. Of the cases where alcohol was detected, 60% had a high back-calculated figure. For the purposes of this paper high is defined as greater than 150 milligrams per 100 millilitres (%). Illicit drugs were detected in 34% of the 1014 cases with cannabis being the most commonly detected drug followed by cocaine, benzodiazepine drugs, opiate drugs, Ecstasy, amphetamine and methadone. A number of samples contained more than one illicit drug. In 2% of cases, a potentially stupefying drug was detected which had not been admitted and therefore could be a genuine DFSA case. GHB was detected in some of these cases but neither flunitrazepam nor its metabolites was detected. Although sedative drugs were detected in many more cases, complainants admitted prescribed use of these medications. A wide range of non-sedative pharmaceutical drugs were also detected. The types and numbers of all these drugs will be presented (together with examples of typical limits of detection).

The results of these studies are in agreement with other studies published in this area. However, this is the first study to our knowledge which has attempted to identify the 'genuine' cases and which discusses in detail the significance of the alcohol levels found in cases of this type.

Keywords: DFSA, flunitrazepam, alcohol

ABSINTHE ANALYSIS: A STUDY OF TOXIC COMPONENTS OF THE NOTORIOUS BEVERAGE

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Introduction: Popular in the 1800's, absinthe is liquor made from the extract of the wormwood plant (*Artemesia absinthium*). The extract had a green hue, which led the drink to be known as the "green fairy". Historically color was enhanced with additives such as antimony and copper, which may have influenced toxic effects. The drink was popular among artists and writers because its inherent hallucinogenic properties known as the absinthe effect. The absinthe effect was known to stimulate creativity and sexual desire. Popularity and excess led to a public health problem, and it was banned in the early 1900's in many countries. There has been a recent resurgence as this ban has been lifted in Europe under new regulations. In countries were absinthe is still banned, orders can be placed over the internet. The major active ingredients are bicyclic terpenes, α-thujone and β-thujone, which are found as diastereoisomers in nature. Thujone is a neurotoxic and porphyrogenic compound. The more potent form is α-thujone, which is believed to block the γ-aminobutyric (GABA) gated chloride channel. The fractional content of α and β-thujone is dependent upon plant source. Current European guidelines limit thujone content to 10 ppm in alcoholic beverages. Analysis was conducted on 5 separate samples to determine thujone and other terpene content, as well as analysis of heavy metal and ethanol content.

Materials and Methods: Five different commercially available absinthe bottles produced in France and Germany were analyzed, each claiming to have an infusion of wormwood plant. Extraction was performed using chloroform as the organic solvent. GC/MS analysis was performed on the extracts using total ion chromatography and selected ion monitoring. A 10 ppm α- thujone standard was used to quantify results (Fluka >96%). Samples were treated with nitric acid, and assayed using ICP/MS to determine the metal content. Ethanol content was assayed on a Roche Integra chemistry analyzer using the alcohol dehydrogenase

Results:

	sample 1	sample 2	sample 3	sample 4	sample 5
α- thujone (ppm)	4.0	1.5	3.4	1.3	0.2
β- thujone (ppm)	2.1	1.2	3.2	0.0	0.0
Total thujone (ppm)	6.1	2.7	6.6	1.3	0.2
EtOH (label) v/v	55%	55%	60%	45%	55%
EtOH (measured) v/v	53%	55%	56%	46%	53%
Copper (ppb)	15.3	71.3	25.8	19.5	19.6
Antimony (ppb)	0	0	6.8	0	0

A number of other related compounds appeared in the samples, which in addition to thujone, are extracts from plant sources used to make absinthe. These were identified with a library spectra match (> 90%). Compounds include camphor, menthol, fenchone, methone, and anisaldehyde.

Conclusion: Modern absinthe does conform to current guidelines in regards to thujone content by our analysis. However, much variation was seen between each manufacturer. One sample did have high levels of antimony, and all had copper in varied concentration. The clinical toxicity of absinthe with high terpene and metal content in the presence of ethanol intoxication needs further research. Although absinthe is still officially banned in the U.S., this product is available on the Internet and popularity is rising. In July 2003, an article appeared in Maxim magazine describing this recent trend and Internet popularity in the U.S. The history of absinthe has shown it to be a public health concern. The high alcohol content combined with the effects of thujone may increase the risk of critical intoxication with consumption of modern absinthe.

Keywords: Absinthe, Analysis, GC/MS

LOW BLOOD ALCOHOL LEVELS, ATTENTIVE FUNCTIONS AND BODY SWAY

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This double-blind study aimed at examining the effects of low blood alcohol levels (BAC) with placebo control and random cross-over distribution. Psychomotor performance and body sway were studied in 16 healthy volunteers after intake of low doses of ethyl alcohol (0.5 g/kg). Neurosensory and psychomotor functions were assessed by a Divided Attention Test (DAT) together with Short-Term Memory and Response Competition Tests (RCT). Changes in body sway were recorded by a stabilometer. Tests were administered at 30, 60, 120 and 180 minutes after alcohol intake. Tests were repeated with a wash-out period of one week. BAC was monitored by breathalyser and analysis of blood levels.

Statistical analysis showed that, as regards the RCT, the number of errors did not increase in a statistically significant way ($p = 0.9071$) in any of the experimental phases after alcohol intake (Table 1). Only the time effect (learning effect) reached statistical significance.

TABLE 1- *Response Competition Test*

Parameter	Treatment		Time		Treatment x Time	
	F	p-value	F	p-value	F	p-value
Errors	0.001	0.9071	10.61	<0.001	0.47	0.7571

Like the RCT, the Memory Test revealed impaired performance after alcohol intake but not at a statistically significant level: p=0.119; Table 2).

TABLE 2- *Memory Test*

Parameter	Treatment		Time		Treatment x Time	
	F	p-value	F	p-value	F	p-value
Repeated words	2.72	0.1199	6.56	0.0002	0.93	0.4537

Stabilometry supplied variations of enormous statistic importance, mainly in antero-posterior and latero-lateral sway (p=0.0001; Tables 3-4).

TABLE 3 - *DAT and Body Sway*

Parameters	t	p-value
□ max-min ant-post body sway	5.8822	0.0001
□ max-min lat-lat body sway	4.1676	0.0001

Careful analysis of the various experimental phases also showed maximum variability in times T1 and T2, i.e., during alcohol absorption.

TABLE 4 - *DAT and Body Sway over time*

Parameters	t	p-value
Baseline: □ max-min ant-post body sway	0.1357	0.8939
T0 : □ max-min ant-post body sway	2.4270	0.0283
T1 : □ max-min ant-post body sway	4.6753	0.0003
T2 : □ max-min ant-post body sway	4.2825	0.0007
T3 : □ max-min ant-post body sway	4.0626	0.0010
Baseline: □ max-min lat-lat body sway	0.9131	0.3756
T0 : □ max-min lat-lat body sway	3.7372	0.0020
T1 : □ max-min lat-lat body sway	4.6520	0.0001
T2 : □ max-min lat-lat body sway	4.8560	0.0002
T3 : □ max-min lat-lat body sway	4.3378	0.0006

The increase in the sensitivity of the body sway test to alcohol-induced alterations in posture during distracting stimuli is clearcut. In conclusions, BACs of under 50 mg% did not reveal statistically significant impairment of memory capacities or motor coordination functions. Instead, statistically significant oscillations in body sway were observed, particularly when the stabilometric test was associated with a distracting stimulus such as the DAT.

Key words: Ethyl alcohol; Psychomotor performance; Body sway.

HUMAN PERFORMANCE FORENSIC TOXICOLOGY IN TURKEY

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Human performance toxicology, also referred to as behavioral toxicology, is a branch of forensic toxicology concerned with the relationship between the presence of a drug and associated behavioral changes. Forensic toxicologists have become interested in human performance toxicology because it is a natural extension of their interest in the medicolegal implications of drug use, misuse, and abuse. This branch needs new educational and analytical approaches and developments in our country. The aim of this study is to describe the present status, progress and applications of human performance toxicology in Turkey.

Forensic Toxicology in Turkey is carried out in the Forensic Medicine Council of the Ministry of Justice and Forensic Medicine Departments and Forensic Medicine Institutes of Universities. Forensic Toxicology is practiced almost exclusively as a result of investigation of fatalities. As a result, postmortem forensic toxicology is the main topic in the Forensic Medicine Council.

In most countries Forensic Toxicology has progressed with the advances in analytical chemistry but the same progress has not been achieved yet in forensic chemistry in countrywide of Turkey except Istanbul Ankara and İzmir. Forensic Medicine Departments that provide education in forensic medicine are also attempting to develop the fields of forensic sciences. There are several forensic toxicology education programs in universities and institutes, including Ege University.

Ege University, in cooperation with the Police Department, is applying on-site (saliva) drug tests in traffic cases. The positive results will be confirmed by Enzyme Multiplied Immunoassay (EMIT) and Gas Chromatography/ Mass Spectrometry (GC/MS). Most countries have legislation covering driving under the influence of drugs (DUID). Most have an impairment approach but not an analytical approach. Several European Union (EU) member states have recently introduced or are preparing 'per se' laws on DUID. Driving under the influence of drugs is prohibited by traffic legislation in our country also, but unfortunately the analytical procedure is not yet effectively used. The Police Criminology Laboratories do not analyze biological specimens for drugs. In routine applications, the impairment approach is only related with driving under the influence of alcohol.

At present, the breath alcohol tests performed by police are routinely applied to motor vehicle operators driving under the influence of alcohol. The allowed ethanol limit for driving under the Turkish Road Traffic Act is 0.5g/L. In alcohol testing, the first suspicion of the police officer is followed by an on-site breath analyzer test and under certain conditions it is followed by the blood test for alcohol in Council of Forensic Medicine cases. Blood Alcohol Determination is performed by Conway- micro diffusion, immunoassay techniques and gas chromatography. Recently, the Forensic Medicine Chemical Analysis Specialty Office has made an effort for analytical toxicological research projects to include GC, GC/MS, High Performance Liquid Chromatography (HPLC) and Head Space Gas Chromatography (HS-GC).

More collaborative projects on educational and laboratory practices will help us to achieve improvements in Forensic Toxicology in Turkey.

Keywords: Human, Performance Toxicology, Turkey

DRUGS OF ABUSE IN PORTUGAL: A STATISTICAL REVIEW

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Introduction: Drug abuse and its eventual association to fatal intoxications, or other violent deaths, is responsible for a great number of requests for drug testing at the Portuguese National Institute of Legal Medicine. With approval of recent legislation in 1998, which establishes procedures for conducting drug screening requested by driving enforcement authorities, it has been possible to develop a new approach to the evaluation of drug consumption linked to driving in situations which did not result in fatal accidents.

Objectives: The authors present a retrospective study involving cases from 2003, from the centre of Portugal. Drug testing in post mortem blood was performed in all cases requested by the pathologist, as well as in those cases of individuals aged less than 50 years old, when the autopsy was inconclusive. The authors also evaluated the incidence of drugs of abuse in deaths related to driving accidents, and discuss the actions of the pathologist, as well as of the law forces, in these situations, regarding the request of the drug screening.

Methods: The samples tested were always whole blood. After screening by enzyme-linked immunoassay (EIA by Coda, Bio-Rad), the positive samples were submitted to a procedure involving solid phase extraction followed by confirmation and quantification analysis using a HP 6890 gas chromatographer equipped with a HP 5973 mass selective detector (GC/MS) using electron-impact ionization. Cut-off values for the most common drugs are established by law: 100, 150, 80 and 1000 ng/mL for opiates, cocaine and metabolites, cannabinoids and amphetamines, respectively. The limits of quantification (LOQ) are less than these cut-off values for all drugs analysed.

Results: The authors present the overall results of testing for drugs of abuse in post mortem samples, with a description of the circumstances of driving accidents. 261 analyses for drug testing in post mortem blood were performed and 18 were positive (6.9%). 61 (23.4%) of the cases were due to driving accidents and only 1 of these was positive for drugs of abuse. Law enforcement authorities requested 163 analyses for drug testing. There were 10 (6.1%) positive results. In 6 of these positive cases, the information was sufficient to establish links to driving accidents.

Conclusion: Since the law that regulates drug screening in driving cases is very recent, it is still difficult to make reliable conclusions about the influence of these substances on driving, and on the incidence of accidents. On the other hand, law enforcement authorities do not consistently request drug testing, because the law is not clear enough in this respect. The pathologists also use variable criteria to request testing, therefore, screening tests for drugs of abuse have only been performed on a small percentage of the total fatal accidents.

Key words: Drugs of abuse, Driving, Portugal

CONCENTRATION OF DRUGS IN BLOOD OF SUSPECTED IMPAIRED DRIVERS

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Blood has been undoubtedly considered to be the only suitable specimen for the evaluation of driving impairment due to drug consumption. In order to gain more information about the type and the concentrations of drugs used by drivers arrested for driving under the influence of drugs (DUID), we analyzed 440 blood samples. All official DUID cases submitted by the Justice Department during a period ranging from 2002 to 2003 concerning living individuals were considered. This study included 400 men (91%) and 40 women (9%). The average age of the drivers was 28±10 years (minimum 16 and maximum 81). One or more psychoactive drugs were found in 89% of blood samples. For the majority of cases (51% from 440), mixtures (from 2 to 6) of psychoactive drugs were detected in blood. The most commonly detected drugs in blood are cannabinoids (54%), ethanol (42%), cocaine (12%), benzodiazepines (11%), amphetamines (8%), opiates (8%) and methadone (6%). Among these 440 cases, THCCOOH was found in 54% (mean 35 ng/ml (1 to 215 ng/ml)), Δ^9-THC in 49% (mean 5 ng/ml (1 to 35 ng/ml)), ethanol in 42% (mean 1,28 g/kg (0.14 to 2.95 g/kg)), benzoylecgonine in 12% (mean 515 ng/ml (29 to 2430 ng/ml)), free morphine in 8% (mean 19 ng/ml (1 to 111 ng/ml)), methadone in 6% (mean 165 ng/ml (27 to 850 ng/ml)), MDMA in 6% (mean 388 ng/ml (10 to 2480 ng/ml)), free codeine in 5% (mean 5 ng/ml (1 to 13 ng/ml)), cocaine in 5% (mean 109 ng/ml (15 to 560 ng/ml)), midazolam in 5% (mean 56 ng/ml (20 to 250 ng/ml)), nordiazépam in 4% (mean 492 ng/ml (30 to 1560 ng/ml)), amphetamine in 4% (mean 63 ng/ml (10 to 183 ng/ml)), diazepam in 2% (mean 279 ng/ml (80 to 630 ng/ml)) and oxazepam in 2% (mean 614 ng/ml (165 to 3830 ng/ml)). Other drugs, such as lorazepam, zolpidem, mirtazapine, methaqualone were found in less than 1% of the cases. Propositions for a new zero-tolerance law will suggest that the presence of scheduled drugs like amphetamine (≥20 ng/ml), methamphetamine (≥20 ng/ml), MDMA (≥50 ng/ml), MDE (≥50 ng/ml), cocaine (≥20 ng/ml), free morphine (≥20 ng/ml) and Δ^9-THC (≥2 ng/ml) is sufficient for prosecution, regardless whether the capacity of driving of the person was impaired. If we apply these conditions to these cases, 60% of the drivers tested in this study would be prosecuted.

Keywords : drugs, alcohol, driving

EFFECTS OF OPIOIDS ON METHAMPHETAMINE-INDUCED STEREOTYPICAL SELF-INJURIOUS BEHAVIOR IN MICE

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Psychostimulants as well as other abused drugs, such as morphine and heroin, induce several behavioral effects in rodents, which is believed to be mediated by the activation of the dopaminergic system. Furthermore, relatively high doses of psychostimulants induce stereotypical self-injurious behavior (SIB) in rodents. The combination of psychostimulants and opioids is quite common and is reportedly used to produce a more intensely pleasurable "rush". Therefore, some investigators have sought to characterize the interactions of these psychostimulants and opioids. However, the effects of opioids on dopamine-related behavior, especially psychostimulant-induced SIB, remain to be fully elucidated. The present study was designed to investigate the effects of μ-, δ- and κ-opioid receptor agonists (morphine (5.0 – 20 mg/kg, s.c.), SNC80 (1.25 – 5.0 mg/kg, s.c.) and U50,488H (1.0 – 10 mg/kg, i.p.), respectively) and buprenorphine (0.125 – 2.0 mg/kg, s.c.) on methamphetamine-induced SIB. After the administration of methamphetamine, SIB, especially skin-picking behavior or self-biting around the chest, was measured for 3 min at 15 min intervals. A score of 0 was given for inactivity of SIB-like behavior, 1 for very mild SIB (less than 1 min), 2 for at least 1 min of SIB, 3 for continuous SIB through 3 min. Methamphetamine (20 mg/kg) induced SIB in 6 out of 7 ddY mice, while in the combination test, the opioid receptor agonists significantly attenuated methamphetamine-induced SIB. The relative potency of these opioids in attenuating methamphetamine-induced SIB was morphine > buprenorphine > U50,488H >> SNC80. These results suggest that the stimulation of μ- and κ- (particularly μ-) opioid receptors exerts an inhibitory effect on stereotyped high-dose methamphetamine-induced SIB in mice.

Keywords: Self-injurious behavior; Methamphetamine; Opioid

LONG-TERM ROADSIDE SURVEYS FOR DRUNKEN DRIVING IN FINLAND

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Numerous reports have shown that the rate of drunken driving declined worldwide in the 1980s. Nevertheless, drunken driving is still a considerable factor in fatal motor vehicle accidents. In 2002, 415 persons were killed in road traffic accidents in Finland (population 5.2 millions). In these accidents the number of fatalities involving drunken drivers was 91 (21.9%), whereas 12 persons (2.9 %) were killed in accidents involving other intoxicants than alcohol. Annual roadside surveys have been carried out since 1978 in the province of Uusimaa in southern Finland to determine the incidence of drivers who had been drinking. The statutory limit of blood alcohol concentration for drunken driving in Finland is 0.5 o/oo (g/kg). The police can make a demand for a driver's breath sample to be tested for the presence of alcohol even in the absence of obvious signs of drunken driving. Annually a total of about 30000 drivers have been breath tested. The surveys have been carried out using the same protocol and, therefore, the results obtained from year to year are comparable. The surveys take place on three Tuesdays and three Saturdays during both spring and autumn. These days are chosen to represent one weekday and one day of the weekend. Every survey consists of 4-5 roadblocks, each lasting 30-40 minutes. The roadside survey team includes one chief inspector, 8-14 police officers, one physician and a few assistants. The driver of every motor vehicle is breath tested with an Alcometer PST-M1R (Lion Laboratories Ltd., Cardiff, U.K.) or an Alcosensor IIIR (Intoximeters Inc., Saint Louis, Missouri, U.S.) screening device. The incidence of drunken drivers has been 0.2 % since the beginning of the 1990s. The percentage of drivers with an alcohol concentration below the statutory limit has been 0.4 % during the 1990s and since 2000 a rising trend has been observed. In 2003 the frequency was 1.01 %. A possible explanation for the observed rise is that the attitude, especially of young drivers, has changed, resulting in an increase in the number of cases involving low blood alcohol concentrations. More and more young drivers recognize that while dining in restaurants or attending parties they can consume one to two beers or ciders while their blood alcohol concentration can remain safely below the statutory limit of 0.5 o/oo. The coming years will show whether the recent significant increase in Finland of drivers with low alcohol content is a permanent trend or not. The taxes of alcohol beverages were substantially lowered in March this year, which may well contribute to maintaining, or accelerating this trend.

Keywords: drunken driving, roadside survey, breath testing

AMELIORATION OF LEAD TOXICITY WITH VITAMIN C AND SILYMARIN SUPPLEMENTS

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The aim of the present study was to investigate the impact of the combined administration of vitamin C and silymarin on lead toxicity. Male albino rats were subdivided into 3 groups; the first was a control group, the second received lead acetate in the diet as 500 mg/Kg diet daily, the third received the same lead acetate dose and supplemented with vitamin C (1 mg/100 g body weight) and silymarin (1 mg/100 g body weight) by gastric tube 3 times per week. Blood samples were taken after 2,4 and 6 weeks of treatment. Significant lead-induced elevations in serum ALT, AST, GGT and ALP activities were observed after different periods of treatment. However serum LDLc was decreased. The intensities of RNA and apoptotic fragments of DNA were measured as optical density by the Gel-pro program. Lead acetate decreased the intensity of DNA at 6 weeks and induced apoptotic DNA fragments reversibly with time. After two weeks of lead administration dilation and congestion of terminal hepatic veins and portal vein branches were observed. Lead also induced hepatocyte proliferation without any localized distribution among zones 1,2,3. Portal inflammatory infiltrate with disruption of the limiting plates (interface hepatitis), steatosis, apoptosis and mild fibrosis were detected especially by sixth week of lead administration. Combined treatment of lead–exposed animals with vitamin C and silymarin showed marked improvement of the biochemical, molecular and histopathological findings. These experimental results strongly indicate the protective effect of vitamin C and silymarin against toxic effects of lead on liver tissue.

Keywords: Lead, vitamin C, silymarin, liver, serum

DFSA-REPORTED INCIDENTS IN THE UK AND THE MELLANBY EFFECT

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A study of 41 cases of drug-facilitated sexual assault (DFSA) in 2003 from 2 separate areas of the United Kingdom has shown some interesting trends. The vast majority (38) of complainants were female, 28 were under 30 years of age, and 30 of the alleged incidents occurred between 21:00 hours and 03:00 hours. Perhaps more significantly, 35 of the cases were alcohol-related. Apart from drugs of abuse (mainly cannabis) found in 16 cases, none of the so-called DFSA drugs (Rohypnol, GHB, sedatives) were detected. These findings echo previously published data (ElSohly et al, 1999) indicating that alcohol is the common denominator in these cases.

The intensity of the CNS effects of alcohol are proportional to the blood alcohol concentration (BAC), as described by Dubowski in 1997, but as noted by Mellanby in 1919, the effects are far more pronounced as the BAC is rising as compared to when the BAC is falling. In addition, as the rate of alcohol ingestion rises, the greater the degree of intoxication (Moskowitz et al 1977).

The number of reported "DFSA" cases in the UK has risen dramatically over the past 5 to 10 years. During a similar period, the UK has seen a steep increase in the availability and popularity of 'alcopop' drinks (bottles of brightly coloured drinks typically containing vodka and lemonade or other fruit flavours, designed to appeal to younger drinkers), plus a wider variety and choice of strong beers, and larger measures of wine and spirits in public houses. There is now government concern and a rising awareness of "binge-drinking" amongst young people in the UK. All of these factors may just be coincidence, but it would appear that changing drinking habits and greater CNS intoxication associated with a rapid BAC rise, might be responsible for the majority of these DFSA allegations.

Key words: DFSA, alcohol, Mellanby

Scientific Session Abstracts:

Clinical and Environmental Toxicology

THE VOLUNTARY DETOXIFICATION PROGRAMME – A SURVEY OF THE EFFICIENCY

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Aims: The object of this survey was to evaluate the effectiveness of our detoxification strategy for future planning and corrections on the basis of the analytical results. Methadone and buprenorphine programme applied to opiate and amphetamine abusers in the detoxification centre for north-east area of the Czech Republic (Hradec Kralove county) is based on the voluntary and liberal principle. However a common problem related to this approach is a relapse to drug abuse. Detection of the key analytes (methylamphetamine, amphetamine,MDMA, MDA, 6-MAM, morphine, codeine, acetylcodeine and cannabinoids, especially 11-nor-delta-9-tetrahydrocannabinolic acid) in urine specimens is the decisive cause of definitive or temporary discharge from the treatment according to the individual status of patient and all case consequencies.

Methods: A total of 3464 urine specimens randomly collected from 85 patients admitted to programme (70 males, 15 females, aged 19 to 43) over an 8-year (1996-2003) period were screened for illicit drug use, especially for opiates and amphetamines. The screening analyses were carried out by immunochemical methods (EMIT II) and full-automatic HPLC-UV procedure (REMEDi HS Drug Profiling System). The confirmations were performed by GC-MS (Magnum MAT Finnigan). A total of 25600 tests were done. Extraction procedures before GC-MS acquisition were carried out by solid phase extraction devices with reagents containing ethyl acetate, acetone, methylene chloride, 0.01 M acetic acid, methanol and ammonium hydroxide. Bond Elute Certify II columns were used for the extraction of amphetamines and opiates after enzymatic hydrolysis with β- glucuronidase at 60^0C for 3 hours. Positive samples for cannabis after deconjugation with 11N potassium hydroxide were extracted on SPEC C18 AR columns. Amphetamines and opiates were detected simultaneously in one analysis in the form of pentafluoropropionyl derivatives (PFPA reagent).Cannabinoids were derivatized with BSTFA + TMCS (99+1) mixture. Samples for evidence of psilocine abuse were extracted on SPEC MP1 mixed disc and derivatized with acetic anhydride in pyridine in the ratio of 3:1 (LOD 10ng/mL). The chromatographic separations were achieved on a DB5ms fused-silica capillary column (30m x 0.25mm x 0,25μm film thickness) using helium as carrier gas at 1ml/min flow rate.

Results: It was found that the commonest illicit drug detected in this programme was cannabis (20%) followed by opiates (9.9 %) and amphetamines (9 %). Other classes of illicit drugs (cocaine, LSD and psilocine) were not detected.

Conclusions: Specimens tested positive for opiates and amphetamines showed decreasing tendency during the whole period of the detoxification programme but specimens tested positive for cannabinoids were stable over time. It seems to be problem concerning the number of positives because positive results for cannabis caused temporary discharge from treatment. In addition to this problem we have to admit that GC-MS such as Magnum does not able to offer sufficient analytical sensitivity for LSD analysis. A cut-off value of LSD immunochemical assay is 1 ng/ml but LOD for our GC-MS procedure was 25 ng/ml after silylation with BSTFA + TMCS, 99 + 1). However, methylamphetamine and heroin users mostly prevailed. Therefore LSD did not belong to main drugs of abuse in this controlled group of abusers. Positive results for LSD above a cut-off value were not obtained. Nevertheless in spite of these facts the analytical data derived from this survey give relative satisfaction and demonstrate the high level of effectiveness of this manner of drugs of abuse therapy.

Keywords: drugs of abuse, substitution therapy, detoxification programme

PARADOXICAL RESULTS FROM SCREENING FOR URINARY 6-AM AND OPIATES WITH DIFFERENT IMMUNOASSAYS IN A HEROIN SUBSTITUTION TREATMENT POPULATION

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Identification of 6-Acetylmorphine (6-AM) in urine samples is regarded as an unequivocal proof of recent heroin abuse. Due to its short elimination half-life of about 30min, 6-AM normally cannot be detected for a longer period than 24 hours after heroin consumption. In addition, it is generally assumed that the metabolite morphine and its glucuronidated forms can be detected much longer. Therefore, the presence of 6-AM in urine samples at detectable levels is only expected together with high total morphine concentrations.

Since the availability of the 6-AM CEDIA (Microgenics) we started to test opiate-CEDIA (Microgenics) positive samples with the 6-AM assay before GC/MS analysis for 6-AM is conducted. This is done semiquantitatively on a Hitachi 911 with calibrators at 0, 5, 10 and 20ng/mL applying a cutoff at 5ng/mL. Quantitative results within the measuring range of the 6-AM CEDIA mostly correlate well with the data of GC/MS but are discrepant when increased amounts of free morphine are present in the sample. This cross-reactivity of free morphine (~0.06%) can even lead to false positive results especially for samples from patients substituted with morphine.

As we always screen urine samples from dihydocodeine substituted patients with the 6-AM CEDIA instead of opiate-CEDIA we wanted to learn if there are any other cross-reactive drugs increasing the 6-AM CEDIA result or can lead to false positives. We therefore run the 6-AM test together with the opiate-CEDIA and opiate-DRI (Microgenics) on every urine sample from substituted patients on 49 consecutive workdays (3696 samples). No false positive results were found with the 6-AM assay. However, in 29 (0.8%) samples from 23 different patients (9 females, 14 males) paradoxical results were observed with positive 6-AM CEDIA results (>5ng/mL) and negative (cutoff 100ng/mL) or low positive results (<400ng/mL) in the opiate tests. The DRI and CEDIA opiate immunoassays gave similar results. Interestingly all the samples analysed were confirmed to be 6-AM positive with only small or undetectable amounts of free morphine. There were only two discrepant samples where the concentration of 6-AM found with GC/MS (9.3ng/mL, 2.5ng/mL) was significantly lower than expected from the 6-AM CEDIA assay (both samples >20ng/mL). An additional cross-reactive substance or perhaps cross-reacting 6-AM-3-glucuronide may be assumed. Ongoing investigations with LC-MS-MS will look for 6-AM-3-glucuronide and morphine-3- and morphine-6-glucuronide in all 29 samples.

Conclusion: Positive screening results for urinary 6-AM as a proof for very recent heroin abuse should be looked at very carefully and always in the context of total morphine concentration.

Keywords: 6-AM, opiates, CEDIA

no.	substitute, dose [mg/day]	creatinine [mg/dL]	6-AM CEDIA >5 ng/mL	Opiates-CEDIA [ng/mL]	Opiates-DRI [ng/mL]	"free" 6-MAM GC/MS [ng/mL]	free Morphine GC/MS [ng/mL]
1	M14	66	16.0	41	42	18.5	1.5
2	M4	91	>20	197	148	30.0	7.9
3	M6	15	>20	85	54	20.7	<0.8
4	M12	40	>20	212	212	9.3	37.0
5	M13	14	>20	81	60	25.0	14.0
6	M8	14	>20	125	119	35.3	41.5
7	M17	52	>20	80	69	58.6	3.1
8	M7	0	>20	217	203	n.f.	n.f
9	M5	106	>20	114	77	19.7	<0.8
10	M12	63	7.5	56	40	10.0	<0.8
11	M10	5	>20	273	251	n.f.	n.f.
12	M6	220	10.9	76	25	n.f.	n.f.
13	M13	90	15.5	68	29	n.f.	n.f.
14	M9	74	8.9	59	40	7.6	<0.8
15	M4.5	100	13.0	99	79	n.f.	n.f.
16	M10	105	>20	147	117	n.f.	n.f.
17	B24	114	20.0	182	172	n.f	n.f
18	M10	47	>20	354	308	45.9	<0.8
19	M6.5	89	7.4	71	23	n.f.	n.f.
20	M12	74	9.6	69	70	n.f.	n.f.
21	M8	90	5.7	110	93	8.0	<0.8
22	M8	351	>20	79	43	2.5	<0.8
23	M7	97	11.0	136	132	n.f.	n.f.
24	M8	185	>20	109	70	n.f.	n.f.
25	M10	108	>20	115	107	n.f.	n.f.
26	M16	14	>20	72	49	n.f.	n.f.
27	M3.5	33	>20	80	55	n.f.	n.f.
28	M4	74	>20	169	151	n.f.	n.f.
29	P2	132	>20	113	75	n.f.	n.f.

M = Methadone
P = Polamidone
B = Buprenorphine

n.f. = not yet finished

ALUMINUM TESTING IN TRACE-METAL FREE CONTAINERS

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The testing of biological samples for elemental analyses can be quit challenging. Specimen collection is a critical aspect of these tests. Proper specimen collection procedures are necessary in ascertaining an analytical result that is truly reflective of the amount of determinant in the specimen. Contamination of the specimen is a considerable problem especially in the analysis of trace metals. The results of analysis of blood and urine specimens can be misleading if proper precautions are not taken to minimize sample contamination. Because of the prevalence of aluminum in our environment, the collection of specimens without precautions can lead to misinterpretation. Contamination may come from the patients themselves, the procedures and devices used in the specimen collection, and/or the laboratory performing the test (including particles and dusts in the air or reagents used in the analysis). Specimen collection tubes are a known source of contamination. The source of the contamination may be from the glass, the stopper, and/or the specimen preservative in the tube. Aluminum testing in biological samples is important for assessing occupational/environmental exposures and for clinical monitoring of renal dialysis patients, among others. The accumulation of aluminum in the body can increase the risk of neurotoxicity ("dialysis encephalopathy"), bone disease and renal failure. This study examined the effectiveness of specially manufactured trace-metal free specimen collection tubes in the determination of aluminum. Specimens were collected consecutively in trace-metal free tubes containing no preservative (serum), and trace-metal free tubes containing either heparin (plasma) or EDTA (plasma) as preservatives. The aluminum concentrations were determined under identical conditions by inductively coupled plasma/mass spectrometry (ICP/MS) with a reporting limit of 5 mcg/L.

A summary of the serum and plasma aluminum findings (mcg/L) is listed below:

Patient	Serum Aluminum	Heparinized Plasma Aluminum	EDTA Plasma Aluminum
A	8	6	11
B	11	13	11
C	5	7	57
D	8	7	6
E	7	13	110
F	8	19	<5
G	12	<5	8
H	8	12	8
I	<5	12	34

These data revealed that most of the findings approximated the reported normal range of less than 10 mcg/L and that by using a one-way ANOVA test of this single patient population there were no statistically significant differences between the types of tube used. However, the three highest aluminum values found (110, 57 and 34 mcg/L) were from patients in which the aluminum concentration was determined from EDTA plasma samples. The increased aluminum found in these three specimens was most likely due to external contamination of the specimens. In each of these individuals, the two other aluminum results (i.e. serum and heparinized plasma) approximated the reported normal range and were comparable, one to the other, with the only unexpected value being from the EDTA plasma sample. The sources of contamination in these specimens appeared to be from the EDTA, but contamination from the collection devices, the collection site and/or the laboratory cannot be excluded. Trace metal free containers should be part of the collection procedures used for all elemental analyses to minimize the potential for specimen contamination. Manufacturers offer evacuated blood-collection tubes especially for trace elemental analysis; however, these tubes are to be used for only those elements that are certified by the manufacturer. For those other less common elements, the manufacturer should be contacted to confirm that the product is suitable for the specific element or the laboratory should verify the absence of the element to be examined before use.

Keywords: Aluminum, Collection, ICP/MS

THE ENVIRONMENTAL IMPACT OF LEAD, CHROMIUM, ZINC AND CADMIUM PRESENT IN RUBBISH DUMP

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Lead, Chromiun, Zinc and Cadmiun concentrations in differents environmental matrixes (soils, drinking underground waters, surfaces waters and edibles fresh vegetables such as celery and lettuce) collected at selected areas near a rubbish dump located at Buenos Aires Province in Argentina were analyzed.

The above mentioned samples were conditioned for their analysis by atomic absorption spectrophotometry. Soils and vegetables samples were first treated by microwave digestion with nitric acid, and then metal concentrations were determinated by aspiration in flame of air- acetylene technique.

Water samples were analyzed by using a graphite oven.

The following table shows the metal concentrations obtained for the different samples :

Samples	Lead ppb	Chromium ppb	Zinc ppb	Cadmium ppb
Underground water 1 (•)	140	70	23	<20
Underground water 2 (•)	10	100	8	<20
Surface Water (▲)	1200	70	ND	ND
	ppm	**ppm**	**ppm**	**ppm**
Vegetables	119	155	68	5
Soil 1	250	34	25	<5

ND – no detectable
(•) about of 2 kilometers of rubbish dump.
(▲) about of 500 meters of rubbish dump

Although these values demonstrated contamination with heavy metals in soils, their respective concentrations did not surpass the recommended values for different uses according to national environmental laws. Nevertheless, the results obtained in edibles fresh vegetables and water showed that there is a high potential of exposure to heavy metals for inhabitants of these areas.

Key words: Environmental Toxicology, Metals Analysis, Underground water, Soils and Vegetables Analysis

DETECTION OF NITRAZEPAM USING ION MOBILITY SPECTROMETRY

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Ion Mobility Spectrophotometer (IMS) has been used as a rapid sensitive and selective technique for the detection of Nitrazepam in body fluids. Nitrazepam an tranquillizer is often misused/abused in criminal activities like theft, robbery, rape etc. The detection of specific compound in body fluid determines the drug administered in such cases. The standard routine procedures are time consuming. In this study IMS was used as rapid technique to detect Nitrazepam. The IMS utilizes the fact that a mixture of different ions may be separated into individual components as they travels at different velocities [mobilities] under the influence of an electric field, through a tube containing the drift gas.

The case of a lady being sexually assaulted and found unconscious was brought to the hospital by police for treatment. The clinical symptoms of being drugged could be observed and the stomach wash collected and sent to the forensic lab for testing. The routine drug screening was followed and simultaneously a portion of the sample was subjected to IMS screening. The ion mobility of standard Benzodiazepines like Diazepam, Flurazepam, Nitrazepam were carried and the drift time and Ko value was noted.

Sl No	Sample	Drift time	Ko
1.	Calibration	9.044	1.86
2.	Diazepam	13.79	1.213
3.	Flurazepam	16.44	1.023
4.	Nitrazepam	16.55	1.016
5.	Sample	16.55	1.016

The exhibit was analysed and the drift time and Ko value could be matched with Nitrazepam. The standard screening procedure of Acidic and basic fraction analysis by GC-MS also confirmed the Nitrazepam. The standard solution of diazepam was studied for the limit of detection with 50 ng solution. The selectivity of the method for the drug identification can be only compared with the standard samples ion motilities analysed sequentially with samples as the drift times of different compounds may overlap on the same. The IMS technique could detect the Nitrazepam even in the gastric lavage directly put on the sample holder of IMS. The interference from the direct analysis had no shift in the peaks. The IMS was found to save time, simple and easy technique to detect drugs of abuse. The procedure can be used to screen for the presence of Benzodiazepine group of drugs in routine analysis and is an additional analytical corroboration in the analysis of drugs.

Key words: IMS, Toxicology, Drug-facilitated sexual assault

MEPROBAMATE OVERDOSE: A ONE-YEAR EVALUATION OF REPORTS

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Meprobamate is a mild sedative-hypnotic drug used in therapy since the 1955s. An overdose with this drug, even taken alone, may cause severe or even fatal respiratory depression, hypotension, shock, and heart failure. This drug is widely known in France, particularly for use in suicide attempts.

One hundred and fifty-three cases of meprobamate intoxication were admitted to the emergency room of our hospital during one year. In these patients, the plasma concentrations of meprobamate measured by gas chromatography after liquid-liquid extractions were above the therapeutic level of 15 mg/L. Ninety-seven intoxicated patients (63%) were female. The age of the patients studied was 14-75 years (mean ± SD, 42.2 ± 12.7). The mean age for females (43.7 ± 13.1) was significantly greater than that for males (39.6 ± 11.6) ($p < 0.05$). Most (58%) of the patients arrived in the emergency room during the time period 6:00 PM to 6:00 AM. Among these 153 hospitalized patients, meprobamate was detected 95 times at concentrations above the toxic level of 50 mg/L. Thirty-two patients (21 %) presented with concentrations in the range of 100-200 mg/L. Six cases of overdose were described with meprobamate levels greater than 200 mg/L; and among them, three voluntary intoxications were fatal (1.96%). One third of the cases turned out to be combined intoxications, 82% of the patients also took benzodiazepines. Most poisonings took place in the first quarter of the year.

In comparison with older studies, the pattern of meprobamate poisoning does not appear to have change in France over the last 25 years. An overdose with this drug, even when taken alone, produces intoxication that is often serious and sometimes fatal. The questionable efficacy of meprobamate as a selective anti-anxiety agent and its potential for life-threatening intoxication are important drawbacks to the clinical use of this drug.

Keywords: meprobamate, overdose, epidemiology

METHCATHINONE: A NEW POSTINDUSTRIAL DRUG

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Methcathinone is an illicit drug also known as ephedrone, which is a methyl derivative of cathinone, a stimulant found in the « khat » plant, *Catha edulis*, and which can be easily manufactured by oxidation from pseudoephedrine. Target consumers of this drug seem to be well-educated people aware of the risks and precautionary measures[1]. Therefore, it is difficult to diagnose and to cure properly and it becomes even more complicated as there are misleading symptoms related to intoxication by cathinone derivatives of synthetic or natural (derived from the khat) origin. Thus far, reports of methcathinone intoxication documented and based on reliable analyses are rare since it is not systematically detected. This paper describes a case of reiterated coma due to an overdose of pure methcathinone dissolved in alcohol and mixed with bromazepam.

Case report: At 10 pm, a 29-year-old woman was admitted in emergency department from Paris suburbs for a coma of toxic origin. Medical history showed that it is the second episode occurring under similar circumstances and at one month interval. In both cases, the patient took Lexomil® dissolved in alcohol. Besides, the family indicated that the patient presented signs of depression, incoherent behavior. She is an amphetamine-like drug consumer. Clinical examination revealed a Glasgow coma score 9 and symmetrical reactive pupils with mydriasis. The patient presented a polypnoea. Her blood pressure was 93/53mmHg. The rest of the examination was unremarkable.

Biological check-up: the ionogram and the blood gas analyses were normal, the blood alcohol level was 1.67 g/L. Urinalysis revealed benzodiazepines, pH 5.4, along with a high concentration of amphetamines dosed by FPIA (Axsym, Abbott – USA) and identified by HPLC (Remedi, Biorad – USA) as follows: methcathinone (17.24 mg/L), ephedrine (11.60 mg/L) and methylephedrine (11.10 mg/L). Serum analysis by HPLC revealed concentrations of bromazepan (8.89 mg/L), methcathinone (0.50 mg/L) and methylephedrine (0.19 mg/L). The patient was kept in quiet room: the hemodynamic and neurological functions evolved quite favourably in the next hours.

Discussion: This case illustrates a coma mainly due to a combined bromazepam and methcathinone intentional intoxication. This coma is not only ascribable to bromazepam and ethanol. Indeed, on one hand, the blood alcohol level at 1.67 g/l, the absence of anionic and basic deficit related to an acetate outbreak eliminates the hypothesis of ethylic coma and, on the other hand, the paradoxical presence of polypnoea instead of respiratory distress eliminates the hypothesis of a coma due to benzodiazepine overdose. Therefore, we can affirm that cathinone altered typical clinical symptoms of bromazepam/alcohol intoxication, namely hypotension and respiratory distress. Methylephedrine detection at infratoxic doses could be a chemical precursor resulting from the alkylation of primary amine for monomethylamine (pseudoephedrine) and dimethylamine (methylephedrine) synthesis (Belhadj-Tahar H. et al. Thérapie, 2003, 59: 265-271). It seems to be chemical tag of a fraudulent origin.

Conclusion: This case describes an example of a new addictive behaviour of "well educated" people involving the intake of methcathinone, a postindustrial psychostimulant intentionally combined with anti-convulsant benzodiazepine.

Keywords: methcathinone, ephedrone, illicit drug

ANALYSIS OF METHYLPHOSPHONIC ACID AND ISOPROPYLMETHYLPHOSPHONIC ACID, A METABOLITE OF SARIN NERVE GAS, BY TANDEM GC MASS SPECTROMETRY AND POSITIVE CHEMICAL IONIZATION

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The two primary metabolites of the nerve agent sarin are methylphosphonic acid (MPA) and isopropylmethylphosphonic acid (IPMPA). They are analyzed by GCMS after derivatization with BSTFA. In positive chemical ionization mode using isobutane, the MH+ ion of the derivatized compound is isolated and then fragmented into product ions for structural elucidation. Blank samples of urine, cleaned up by solid phase extraction, were spiked with commercially available MPA and IPMPA. The linearity and detection limits of the method were determined in urine using IPMPAd7 as an internal standard. Multiple scan events were set up to perform sequential Full Scan and MS/MS. The linear fit for MPA-TMS was R^2= 1.000 (Full Scan) and 0.9982 (MS/MS/MS) and for IPMPA-TMS, R^2 = 0.9990 (Full Scan) and 0.9978 (MS/MS) with a precision of 5.1% RSD for the internal standard (IPMPA-d7-TMS). The precision for the internal standard for 60 replicate injections of spiked urine was 9.5 %RSD. The instrument of analysis was the external source quadrupole ion trap with a temperature programmable injector. Mass Frontier software was used to predict the MS/MS product ions for assistance in the selection of a precursor ion of the derivatized target compounds.

Keywords: Ion trap, MS/MS, nerve gas

A PROPOSED SCHEME FOR FOXY METABOLISM

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We report an emergency room admission following an ingestion of 5-Methoxy-N,N-diisopropyltryptamine, 5-MeO-DIPT or FOXY. The subject was a 23 year old Caucasian male presenting 3 hours post ingestion of a capsule provided by an acquaintance and initially described as "acid". This ingestion was preceded by intake of 4 beers. Approximately thirty minutes before presentation the subject experienced four episodes of vomiting followed by tactile hallucinations and paranoia, primarily related to a suspicion of being poisoned. He denied auditory and visual hallucinations or other neurological symptoms. Vital signs revealed a temperature of 37 °C, pulse 76 bpm, respirations 18 per min and blood pressure of 135/70 mm Hg. Pupils were midpoint and reactive with normal neurological, motor, reflex, cardiovascular and gastrointestinal function. Blood and urine chemistry and hematology results were not revealing. Urine toxicology screening reported presence of 5-MeO-DIPT and suspected metabolites. The subject received activated charcoal, intravenous fluids, three additional hours of supportive care with resolution of his initial condition and was discharged without complaints or subsequent readmission.

Urine toxicology testing involved initial screening by ToxiLab® (Varian Inc, Lake Forest CA) followed by confirmation by electron impact GC/MS. Toxi-A revealed three spots at RF 2.4, 4.6, 6.1 (S1 all blanch, S2 4.6 tan, others faded, S3 all UV absorption, S4 all brown). Extraction for confirmation was preceded by combination of 2 mL of urine, 0.5 mL of 1 N sodium hydroxide, 500 ng of internal standard (SKF-525-A) and 5 mL of dichloromethane. Mixing, separation of the organic layer, evaporation under an air stream at 45 °C in a water bath was followed by reconstitution with 25 µL of ethyl acetate. One µL of the extract was injected for GC/MS analysis on a Hewlett Packard 5972A Mass Selective Detector equipped with a 30 m, 0.25 mm id DB-5® (Agilent Technologies, Wilmington DE) capillary gc column with a 0.25 µm film thickness using splitless injection. Oven temperature was initially 120 °C for 3 min, raised to 225 °C at 10 °C per min and held for 5 min, followed by a second temperature increase to 300 °C at 15 °C per min and a 3 min hold.

Electron impact GC/MS data revealed four chromatographic peaks (RT 15.86, 18.76, 20.13, 21.07) with molecular ions of 232, 274, 260, and 290 m/z, respectively. These have been tentatively identified, in chromatographic order, as 5-MeO-NIPT, the N-desalkyl metabolite, 5-MeO-DIPT, the parent substance, 5-OH-DIPT, the O-desmethyl metabolite, and 5-MeO-DIPT N-oxide, the ring oxidation product of the parent substance. Identifications were based on comparative spectra with literature sources and, in the case of 5-OH-DIPT, corroborative CI/MS with 5 % ammonia in methane, and ethylation with ethyl iodide/TMAH. Assuming that peak heights reflect urine concentrations, the relative magnitude of parent substance and metabolites were 5-MeO-DIPT>5-OH-DIPT>5-MeO-DIPT N-oxide>5-MeO-NIPT. Detection and identification of these metabolites permits the characterization of FOXY metabolism as three parallel oxidative pathways involving each of the molecule's non-carbon sites.

Key words: 5-Methoxy-N,N-diisopropyltryptamine, metabolism, FOXY

EFFECTS OF CHRONIC ACONITINE ADMINISTRATION ON ITS CONCENTRATION IN LIVER, KIDNEY, HEART, AND BLOOD OF MICE

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Aims: Aconitum alkaloids are well known for their acute and high toxicity, for example, in the causation of severe arrhythmias leading to death. Aconitine, one of the major *Aconitum* alkaloids, is a highly toxic compound from the *Aconitum* species. The use of *Aconitum* alkaloids has been known since ancient times, over 2000 years in Asia as a homicidal agent, sometimes as poison for arrowheads. Even today, aconites are sometimes used as homicidal or suicidal agents. Until today, several cases of murder have been reported using single-dose of a large amount of *Aconitum* alkaloids in Japan. In 1995, a man murdered was subjected to autopsy in which *Aconitum* alkaloids were administered repeatedly over a period of months, in Saitama Prefecture, Japan. Although there are various studies reported on the single-dose effect of aconitine, no reports are available on the long-term effects of aconitine, probably due to its high toxicity. Therefore this study was conducted to investigate the influence of chronic administration of aconitine in experimental animal models.

Methods: A total volume of 1.0 mg/kg/day was administered to the experimental animal models. The lethal dose 50% (LD50) of aconitine for mice is 1.8 mg/kg (orally, single dose) and 0.308 mg/kg (intraperitoneally, single dose). The male ICR (Institute of Cancer Research) mice were divided into 2 study groups: "acute group" (day 0: 0, 15, 30, 60, 90, 120 min, 1440 min = 24 hours) and "chronic group" (days 1,3,7,10,15,19,and 22), according to the time when the animals were sacrificed. The experiments were conducted according to the guidelines of the Ethical Committee on Animal Experimentation of Nippon Medical School (Tokyo, Japan). We determined the concentration of aconitine and its metabolites (benzoylaconine and aconine) in organs and blood with gas chromatography/selected ion monitoring (GC/SIM). In addition, we concurrently recorded the electrocardiogram (ECG).

Results: Fifteen min after administration on day 0, the early aconitine administered group (acute group) revealed peak organs and blood concentration levels of aconitine with gradual decrease, thereafter. The concentration of aconitine in organs and blood (from day 0 to day 22; 90 min after the last administration of aconitine) gradually decreased according to repeated administration, whereas benzoylaconine and aconine increased. ECG revealed various types of arrhythmias (for example, ventricular fibrillation, ventricular tachycardia, torsade de pointes, atrioventricular block, and bundle branch block). However, the frequency of arrhythmias remarkably decreased with time and repeated administration of aconitine.

Conclusions: In this study, 2 facts were revealed. First, the frequencies of fatal arrhythmias remarkably decreased to day 22. Secondly, the organs and blood concentration of aconitine (90 min after the last administration of aconitine) gradually decreased and its metabolites (benzoylaconine and aconine) increased until day 22. These 2 facts have raised the possibility that the activity of drug metabolism increased due to long-term administration of aconitine. In the case of long-term administration of aconitine, it is very important to determine not only the concentration of aconitine but also its metabolites (benzoylaconine and aconine) in the organs and blood from the viewpoint of forensic toxicology.

Keywords: aconitine; chronic administration; GC/SIM

ABDOMINAL COMPLICATION OF INHALED METHAMPHETAMINE

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Methamphetamine toxicity continues to be the leading cause of referral to the poison center. Despite the abundance of information regarding its cardiovascular and central nervous system toxicity; abdominal complications related to inhaled methamphetamine are not commonly encountered and anticipated. We report a case of a 33 year old female, a known methamphetamine abuser for the last five years who consulted the emergency room for acute onset of severe abdominal pain and dysuria noted a day prior to consult. Gynecologic problem was ruled out. Abdominal ultrasound showed bile sludge in the gallbladder. She underwent emergency exploratory laparotomy under general anesthesia. However, intraoperative findings were normal. The initial urine methamphetamine level done on the fifth day after the last used showed 2157 ng/ml. This report suggests that methamphetamine toxicity could present as "acute abdomen" and should be anticipated by emergency room physicians.

Key words: Inhalation, Methamphetamine, Abdomen pain

TOXICOKINETICS, RECOVERY, RESIDUES AND CYTOTOXICITY OF ACTP-ESTER IN THE GOAT

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ACTP- Ester (Triclopyr butyl) marketed by Dow Elanco, USA is a selective herbicide used widely in agricultural field. In order to study the toxicokinetic, total recovery and cytotoxicity study of ACTP- Ester and its two potential metabolites - triclopyr acid (M_1) and 3,5,6- trichloro-2 pyridinol (M_2), the compound ACTP- Ester was administered orally 396 mg/kg body wt. to Black Bengal Goats (*Capra capra).* The control groups were however treated with the same amount of carboxymethyl cellulose (CMC).

Blood samples were collected before (0 hr) and after 0.25, 0.50, 0.75, 1, 2,3,4,6,8,12,36,48,60,72,84,96, 120, 144 and 168hr post administration (Pd) and were prepared for HPLC analysis following standard protocols. Toxicokinetic parameters of ACTP Ester were determined from computerized interactive curve fitting programme of respective blood level time profile and data were analyzed as per the standard formula using various disposition kinetic parameters. Gross lesions for different tissues like liver, kidney, lung, brain etc were recorded for ACTP-Ester induced cytotoxicity in goats sacrificed on 4, 5, 6 and 7-day pd. An adequate blood level of ACTP- Ester was detected as 4.94 ± 0.43 at 0.25 hr (pd). The concentration (C^B max 25.15 ± 2.62 µg /ml was recorded at 6 hr followed by a slow decline and minimum (C^B min 3.52 ± 0.23 µg /ml) was detected at 60 hr pd. Kinetic behaviour of ACTP- Ester in goats followed a "two compartmental open model kinetics". The blood level for M_1 and M_2 was 6.52 ± 0.89 and 2.61 ± 0.13 µg/ml respectively. The lower absorption rate constant suggested slow absorption of ACTPEster from G.I.tract. The CI_H value, which is equivalent to CI_B value suggested that major route of elimination of ACTP-Ester be directed through liver. Very poor CI g value suggested that urine is the minor route of excretion of the compound. The concentration of both metabolites appears zigzag fashion, which might be due to variable quantity as well as rate of metabolism in every unit of time.

The recovery percentage of ACTP -Ester from faeces, G.I.tract content, urine and tissues were respectively 65.38, 63.34, 66.10 and 66.24 in goats sacrificed on 4, 5, 6 and 7-day pd. Liver and lung showed gross malformations than other tissues due to the treatment.

Keywords: Toxicokinetic, residue cytotoxicity ACTP, goat

A HOMOGENEOUS ENZYME IMMUNOASSAY FOR THE DETECTION OF OXYCODONE AND OXYMORPHONE IN URINE

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Oxycodone is an opioid analgesic derived from thebaine. It is structurally similar to codeine with a higher abuse potential than morphine. Oxycodone is prescribed for the treatment of moderate to severe pain, chronic pain or terminal cancer pain and is available as OxyContin® or in combination with acetaminophen or aspirin. Oxycodone is metabolized to oxymorphone, an active metabolite that has the same analgesic potency as oxycodone. Other metabolites of oxycodone include noroxycodone and noroxymorphone, which are inactive. The majority of oxycodone is excreted in urine within 24 hours as free oxycodone, conjugated oxycodone and oxymorphone. Increased use of oxycodone has resulted in an increase in oxycodone abuse and abuse-related deaths. None of the currently available opiate immunoassays has the sensitivity to detect oxycodone or oxymorphone at therapeutic or abuse levels. The objective of this study is to develop a homogeneous immunoassay for the specific detection of oxycodone and oxymorphone at therapeutic as well as abuse levels in urine.

Microgenics DRI® Oxycodone Assay is a dual cutoff assay using 100 ng/mL and 300 ng/mL oxycodone as cutoff calibrators. The assay uses a highly specific monoclonal antibody that can detect both oxycodone and oxymorphone. The assay is based on competition between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug from urine sample for a fixed amount of antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the enzyme labeled drug causing a decrease in enzyme activity. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring its ability to convert NAD to NADH.

The reagents and calibrators are liquid ready-to-use. The dynamic range of the assay is 0 to 1000 ng/mL. The performance of the assay was evaluated on the Hitachi 717 analyzer. The within-run and total precision (CV) for the cutoff calibrators and ± 25% controls is <1.0% (qualitative) and <5.0% (semi-quantitative), respectively. The limit of detection is 4.9 ng/mL. The recovery studies yielded recovery ±10% of expected values. The assay has 100% cross-reactivity to oxycodone and oxymorphone with minimal cross-reactivity to opiate compounds. In addition, the assay detects glucuronide conjugates of both oxycodone and oxymorphone, as evidenced by a comparison of enzyme immunoassay results with GC/MS results of samples with and without glucuronidase enzyme hydrolysis. This study also indicated that most of the oxymorphone in urine samples existed as conjugated oxymorphone and free oxymorphone is rarely detected in samples without enzyme hydrolysis. One hundred and seventy samples were tested by immunoassay and GC/MS. All the samples for GC/MS were hydrolyzed with enzyme glucuronidase. Sample correlation studies with GC/MS yielded a Deming's regression y=0.990x+1, with a correlation coefficient of 0.971. The immunoassay results showed >95% agreement with GC/MS.

DRI Oxycodone Assay demonstrated excellent specificity and sensitivity to oxycodone and its major metabolite oxymorphone. The assay is a convenient method that can be applied to various clinical chemistry analyzers.

Keywords: Oxycodone, Oxymorphone, Enzyme Immunoassay.

COMPARATIVE STUDY ON TOXIC MANIFESTATIONS INDUCED BY INGESTION OR INJECTION OF COMMONLY USED DISINFECTANTS AND SURFACTANTS

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Background and aim: Accidental or intentional ingestion or injection of household products sometimes occur due to their easy accessibilities, but the toxic manifestations have not been well characterized when they are internally administered since these products are not developed for medicines. We previously evaluated the toxic and kinetic properties of Osvan® (benzalkonium chloride) that is widely used as a cationic surfactant and disinfectant, in which we showed that (1) different toxicological progression and manifestation appeared in administration via femoral artery (FA) among intravascular administrations even though the blood concentration profiles were similar, and (2) the degree of toxicity correlated with the peak blood concentrations in orally dosed (PO) rats (Toxicol Lett 148: 113-123, 2004). The aim of this study was to evaluate whether or not the difference in toxic manifestations among routes of administration observed in Osvan® were specific only to cationic surfactant, any kinds of surfactants, or any kinds of disinfectants.

Materials and Methods: The test drugs involved Osvan® (cationic surfactant and disinfectant), Hyamine® (cationic surfactant and disinfectant), Tego® (zwitterionic surfactant and disinfectant), linear alkylbenzene sulfonate C_{12} (LAS_{12}) (anionic surfactant), Volpo®20 (nonionic surfactant), Maskin® (non-surfactant and disinfectant), Ethanol (non-surfactant and disinfectant) and saline (control). Fifty-five male Sprague-Dawley rats were administered one of the test drugs orally, intravenously via jugular vein (JV) or intraarterially via FA. Two to four different doses of drug were examined for each drug, and varied between 3 to 150 mg/kg for a intravascular dose based on individual LD_{50} and 250 or 1250 mg/kg for a oral dose except for ethanol. The dose of ethanol was set up at 0.3-1.5 g/kg and 1.5-7.5 g/kg for a intravascular and oral dose, respectively. The condition of rat was observed for 24 h after a dose and then the rat was sacrificed. The cardiac blood and tissue samples were collected for assay and histological examination. The rats that died before 24 h were autopsied immediately after death to collect samples.

Results and discussion: Toxic manifestations were different among the routes of administrations within the same test drugs except for Volpo®20 and saline. The toxic peak appeared soon after the dose following JV administration, while toxic effects developed with the lapse of time following higher doses of FA and PO administrations, though the degree of effects or time-course symptoms varied among drugs. Necrotic-like symptom developed around the injecting side of leg following FA administration in cationic or twitterionic surfactants and Maskin®, while it appeared on opposite side of leg in LAS_{12}. The FA-dosed rats had higher blood myoglobin concentrations compared to JV- or PO-dosed rats and they hardly urinated after a dose, suggesting that kidney was highly affected in FA administration. In PO administration, all high dose of surfactants except for Volpo®20 died at 5-20 h after a dose, while all non-surfactant disinfectants survived for 1 day at the same dose, suggesting that ionic surfactants have greater toxicities in PO administration. Severe damage of internal membrane of alimentary tract observed in ionic surfactants indicated that the ionic surfactant is critical chemical property to induce toxic effects in PO administration. The overall toxic degree based on the dose-size, except ethanol, could be ranked as strong as cationic surfactant = Maskin > zwitterionic surfactant = anionic surfactant > nonionic surfactant. These results suggested that (1) generally ranked stronger disinfectants have higher toxicities when they are intravascularly administered, (2) toxic manifestations are different between JV and FA administrations but with having similar trend in any kind of disinfectants and surfactants, and (3) ionic surfactants have stronger toxicities compared to nonionic surfactant or non-surfactant when they are orally administered.

Keywords: surfactant, disinfectant, toxicity

RAPID DETERMINATION OF CAUSATIVE AGENT USING DETACHED ROOF OF BULLA IN CHEMICAL BURNS OR DERMAL EXPOSURE

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Background and aim: Liquid specimens such as blood or urine are commonly used in forensic examinations, while skin samples are rarely used. Recently we have been studying the percutaneous absorption of chemicals and have shown that skin analysis is useful in identifying dermal exposure to petroleum products. Only small amounts of skin (<0.03 g) are required for analysis since lipophilic chemicals, such as aliphatic hydrocarbons used to identify the petroleum product, remain in the skin (Forensic Sci Int 133, 141-145, 2003). However, most living patients do not agree to have a skin biopsy for chemical analysis. We present a case of a patient who was exposed dermally to some solvents where we determined the causative agent without skin biopsy.

A case: A 73-year-old woman was first aware of slight redness with soreness on her anterior right thigh one evening. When she took off her trousers 3 h later, the redness had spread over her right thigh up to her abdomen with some erosion. When she visited our hospital next morning, extensive erythema with bulla and erosion appeared over 10% of her body. These first- and second-degree burns were thought to be caused by exposure to some unidentified organic solvents.

Examination: The lesions were first washed thoroughly with saline. To examine the lesions pathologically, and to identify the causative agent, a skin biopsy was recommended but not agreed upon with the patient. For an alternative specimen, small pieces of detached roof of bulla, which are usually taken off, were collected for analysis during topical treatment with a steroid ointment. For rapid examination to estimate the causative agent, a part of the collected bulla (0.01 g) was put into small amount of *n*-pentane in a glass tube, sonicated in an ultrasonic bath for 1 min, and then 1 µl of *n*-pentane was analyzed using a GC-MS system (HP5890) in a scan mode (m/z 30-400) equipped with a capillary column (HP-5, 0.25 mm i.d. × 30 m, 0.25 µm thickness). The column temperature was set at 50°C for 1 min, then increased 10°C/min up to 280°C and held for 10 min. The temperature of injection port and ion source was set at 270°C and 280°C, respectively.

Results: In the rapid analysis, typical kerosene components (aliphatic hydrocarbons with carbon number 9-16 and some aromatic hydrocarbons) were detected based on the retention times, mass spectra and pattern of the peaks. The causative agent was determined kerosene. Detailed analysis performed later using the remained collected roof of bulla and blood sample (0.5 ml) utilizing liquid-liquid extraction confirmed kerosene components in the both samples. No other chemicals causing inflammatory response were detected.

Discussion: The patient may have had been exposed to kerosene while filling a kerosene stove at home about 5 h before first becoming aware of her skin irritation. Subepidermal bulla, often observed in second-degree burns is a phenomenon separating epidermis from dermis, indicating that the detached roof of bulla in this case was composed mostly of epidermis. We recently reported that lipophilic chemicals tend to be trapped in lipophilic stratum corneum in epidermis (Int J Legal Med 118, 41-46, 2004). This suggests that a skin biopsy, which removes skin to the subcutaneous fat depth, and causes significant patients pain, is not necessary when petroleum products are involved. Analysis of only 0.01 g of detached roof of bulla with simple sample preparation is a useful diagnostic method for dermal exposure to petroleum products both in clinical and forensic fields.

Key words: chemical burns, bulla, GC-MS

A CASE OF TETRODOTOXIN POISONING CAUSED BY INSERTION OF A PUFFER FISH INTO THE VAGINA

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We report an unusual case of sudden heart and pulmonary failure in a 33 year-old young woman due to insertion of a puffer fish into the vagina. While traveling on a boat, the woman felt unwell while having sex with a man who had inserted a puffer fish into her vagina. Upon arriving at port, she became unconscious and then went into cardio-pulmonary arrest. She was immediately transported to a hospital where she received cardio-pulmonary resuscitation. Following an intravenous injection of 2 mg of epinephrine, her heart rate and blood circulation recovered. Few days later, she began spontaneous breathing, however, her level of consciousness continued at a low stage (JSC 200). She remained in a vegetable state for two months. Following her transfer to another hospital, she died of sequential pneumonia two months later. A serum specimen collected upon admission and a urine specimen collected two days post-admission were found to contain 58 ng/g and 65 ng/mL of tetrodotoxin (TTX). TTX in serum and urine was hydrolyzed to 2-amino-6-hydroxymethyl-8-hydroquinazoline (C_9-bas)e by NaOH. C_9-base was purified by C_{18}-SPE cartridge and analyzed by GC/MS after TMS derivatization. It is known that some species of puffer fish release TTX from the skin. In this case, a toxic concentration of TTX was rapidly absorbed by the victim's vaginal membrane, distributed throughout her body causing her cardio-pulmonary arrest and eventual death.

Key words: puffer fish, tetrodotoxin, vagina

CHANGES OF GENE EXPRESSION BEFORE AND AFTER ACETAMINOPHEN INJECTION DETECTED BY DIFFERENTIAL DISPLAY

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Objectives: Acetaminophen is a familiar antipyretic analgesic. While the mechanism by which acetaminophen causes chemical damage has been fully explained, the molecular biological dynamics that takes place in the damaged liver has not been elucidated. The current study was conducted to advance the molecular biological understanding in the dynamics of the gene expression during the subsequent hepatic damage by acetaminophen.

Materials and Method: 1)Preparation of mice with acetaminophen-induced hepatic damage. Following fasting for 24 hours, 250 mg/kg body weight of an acetaminophen solution was administered via a peritoneal route to mice to serve as a model for a drug-induced hepatic disorder, while the same amount of a physiological saline was also given via the peritoneal injection to the control animals. Both groups fasted for an additional 24 hours thereafter. Under chloroform anesthesia, laparotomies were performed. Following blood specimen collection from the heart, the liver was excised. 2) Serum ALT and AST determinations were measured by using an autoanalyzer (Hitachi-7450). 3) The excised livers were frozen with liquid nitrogen and pulverized, from which RNA was collected by using Trizol. The product was then treated with DNAse and processed with phenol chloroform for cDNA synthesis. The DNA was amplified by using a primer from the Fluorescence Differential Display Kit (Takara). 4) The second PCR and acrylamide gel electrophoresis were conducted by employing the fluorescence differential display method. After a band pattern was visualized by a molecular imager FX, the bands with eminent differences were cut out, from which the amplified products were recovered for cloning. 5) The homology was confirmed between each base sequence that had been obtained from the Web site NCBI BRAST and the known mouse cDNA.

Results: The model for a drug-induced hepatic disorder displayed numerous small white blotches, which were visible with the naked eye, around the hepatic portal system; and the results of blood chemical analyses indicated AST and ALT levels that were significantly higher ($P<0.05$, Student t-test) than those of the control. These findings confirmed that a model for a drug-induced liver disorder had been successfully created in these animals by administering acetaminophen. When the RNA fingerprints obtained via the fluorescent differential display method were compared, evident differences were noted in 69 out of 216 lanes or 107 types. Among these, the expressed genes appeared to have increased quantitatively in 39 lanes or 67 types and quantitative decreases were suggested in 30 lanes or 40 types. From these, 69 lanes or 79 types were cut out and sequence analysis was conducted on 28 lanes or 40 types, among which homology with known mouse cDNA was confirmed in 14 types. The genes that were obtained from the bands with suspected quantitative increases in the expressed genes were: cytochrome P450, 4a10 (Cyp4a10), mitochondrial DNA, mannosidase 2 alpha 1 (Man2a1), TTF-1 interacting protein 5 (TIP5 gene), methylthioadenosine phosphorylase (mtap), suppressor of actin mutations (SAC1 gene), and RIKEN cDNA 4930579A11. Those genes that were obtained from the bands with suspected quantitative decreases in the expressed genes were: cytochrome P450, 4a10 (Cyp4a10), cytochrome P450, 2c37 (Cyp2c37), mannosidase 2 alpha 1 (Man2a1), and suppressor of actin mutations (SAC1 gene).

Discussion: The mRNA for active metabolites of acetaminophen (CYP2E1 and CYP1A2 of P450) was expected to increase but the results of the current study did not support this expectation. It is possible that the molecular deviations recognized in the present experiment reflect the stress that is caused by inflammation or drug administration as well as by compensatory reactions to such stresses and that they represent specific changes caused by acetaminophen.

Keywords: Acetaminophen: Differential display: Pharmacogenomics

EMBLICA OFFICINALIS (FRUIT) INHIBITION OF LIVER FIBROSIS INDUCED BY CARBON TETRACHLORIDE AND THIOACETAMIDE

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The study was carried out to investigate the antifibrotic effects of hydro-ethanolic extract of a reputed Indian medicinal plant Emblica officinalis fruit on liver fibrosis induced by carbon tetrachloride (CCl_4) and thioacetamide in rats. Liver fibrosis was assessed by measuring levels of hydroxy proline, lipid peroxidation (LPO) based on malondialdehyde (MDA) production, microsomal drug metabolizing enzyme CYP450 2EI measured as aniline hydroxylation, along with the antioxidant enzyme systems as catalase (CAT), Glutathione peroxidase (GSH-Px), Glutathione (GSH). Liver $Na^+ K^+$ ATPase and γ-glutathione transpeptidase (γ-gt) were studied to assess the cell membrane disintegration together with serum enzyme activities (AST, ALT, ALP, Bilirubin). In both models of chemically induced liver fibrosis, the levels of hydroxyproline, LPO, CYP450 2EI, $Na^+ K^+$ ATPase, γ-gt along with serum enzumes remained significantly increased with suppression of anti-oxidant defence enzymes. Treatment with a hydro-ethanolic extract of Emblica officinalis significantly reduced the levels of hydorxyporline, MDA, , $Na^+ K^+$ ATPase, γ-gt and serum enzymes. The anti-oxidant defence enzymes were restored to normal values as well. Thus this study finds Emblica officinalis as a useful alternative source of treatment in chronic liver fibrosis.

Keywords: Liver fibrosis, *Emblica officinalis*, rat

A CASE OF SURREPTITIOUS NON-FATAL MERCURY POISONING

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A case of non-fatal mercury poisoning disclosed suspicion of criminal poisoning and resultant toxicology testing is presented. A 46 yr-old, white male Fire Chief with episodes of chest pain and diaphoresis, presented in the emergency department with a one month history of fatigue, generalized weakness, headaches, "inability to focus at work" and "hardly able to walk up stairs", but denies shortness of breath. He had a prior history of episodes of chest pain and diaphoresis. Physical examination and chest X-rays were unremarkable, serum chemistries and enzymes including CPK and LDH, and thyroid were normal. He displayed an EKG on normal rhythm. His hospital course was uneventful and he was discharged after four days. Five weeks later, the chief once again presented in the emergency department with a four day history of: "chills and shakes", a fever of 102^{O}F, back pain, headaches, increasing fatigue and muscular weakness, tingling in his fingers and toes and a palpable tender left axilla lymph node. Once again the physical examination was unremarkable and laboratory test normal with the exception of a serum creatinine of 1.5 mg/dL and a urine protein of 60 mg. His hospital course was uneventful and he was discharged after three days. Three weeks after discharge while eating at work a lunch provided by his assistant-chief, the chief began vomiting violently. Suspicion of poisoning was raised by the comments of co-workers. Therefore, coffee prepared the next morning for the chief by his assistant was saved and given to the police. Initial screening of the coffee for toxic metals by the Reinsch Test resulted in a classic "silver mirror" indicating the presence of mercury. The coffee and a 24 hour urine specimen obtained from the chief were then analyzed in a "cold vapor" Mercury Analyzer System (Perkin Elmer Corp.). The urine contained 20ug of mercury/gm of creatinine. While executing a search warrant at the assistant-chief's home, the police found over 128 reagent chemicals and explosives, including several mercury compounds. The assistant admitted adding mercurous nitrate periodically to the chief's coffee, to sandwiches the day the chief was violently vomiting, and to doughnuts the day of the chief's first emergency room admission. The motive for the poisoning was that the assistant had been passed over for promotion. Three years prior to the presented case, a former chief working with the assistant, was retired with "complete disability" due to a neuromuscular disease of unknown etiology characterized by slow mental functioning, peripheral neuropathy and chronic nephritis! This case exemplifies the difficultly of diagnosing the rather diffuse and non-specific signs and symptoms of surreptitious chronic mercury poisoning.

Keywords: Mercury poisoning, criminal poisoning, mercury analysis

INVESTIGATION OF THE ACCUMULATION OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN RAT KIDNEYS

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A study was conducted to assess the accumulation in the kidneys of 2,4-dichlorophenoxyacetic acid (2,4-D), the most widely used herbicide in the world, and its metabolite 2,4-dichlorophenol (2,4-DCP). 2,4-D is eliminated in humans and animals mainly through renal excretion. Male and female Sprague-Dawley rats were treated with 2,4-D that was introduced in their drinking water for 30 days. Group A (control group) was fed a normal diet, while Group B was treated with 50 ppm, and Group C 100 ppm 2,4-D. In addition, 2,4-D was given daily as an oral dose, combined with their drinking water, consisting of 25 ppm in Group D and 50 ppm in Group E. Levels of 2,4-D and its metabolite 2,4-DCP in the kidneys were measured using an HPLC method. It was observed that, though the administered doses of 2,4-D did not produce significant toxic effects, its metabolite in particular was present at high levels in the kidneys.

Keywords: 2,4-dichlorophenoxyacetic acid (2,4-D); 2,4-dichlorophenol (2,4-DCP); HPLC

RAPID, SIMPLE AND VALIDATED GC-MS ASSAY FOR DETERMINATION OF DRUGS RELEVANT IN DIAGNOSIS OF BRAIN DEATH IN HUMAN BLOOD PLASMA

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Background: Before declaring the brain death of a patient (e.g. prior to explantation of organs), a number of requirements have to be fulfilled. One of them is the exclusion of effective plasma concentrations of drugs which might mimic brain death, especially of those typically administered in intensive care medicine. Recommendations for toxicological analysis in the context of the diagnosis of brain death have recently been published (Hallbach et al., TIAFT Bulletin 34, 2004, 14-16), which include a minimum consensus on the relevant analytes (thiopental, pentobarbital, methohexital, phenobarbital, diazepam, nordiazepam, and midazolam). The proposed limits of quantification correspond to one half of their lowest therapeutic concentrations. Therefore, the aim of the presented study was to develop and validate a rapid and simple assay for determination of the above mentioned drugs in human plasma samples.

Methods: After addition of 50 µl of internal standard solution (4.0 mg/l pentobarbital-d_5, 2.0 mg/l methohexital-d_5, 40.0 mg/l phenobarbital-d_5, 0.8 mg/l diazepam-d_5, and 0.8 mg/l nordiazepam-d_5 in butyl acetate) and 50 µl of butyl acetate to 200 µl of plasma, the samples were extracted for 2 min on a rotary shaker. After phase separation by centrifugation (1 min, 10 000 *g*), 2 µl of the organic phase (upper) were injected into the GC-MS system (Agilent, GC-MSD 5973). The analytes were separated within 10 min by gas chromatography (HP-1 column, 12 m × 0.2 mm I.D.) and detected by mass spectrometry. The mass spectrometer was operated in the full scan mode for identification and in the selected ion mode (SIM) for quantification (target ions *m/z* 156, 161, 172, 247, 252, 204, 209, 256, 261, 242, 247, 310). Validation was performed according to a minimum consensus on method validation in this context currently developed by the Clinical Toxicology Committee of the GTFCh. This included evaluation of selectivity, calibration model, precision and accuracy. Furthermore, the results for accuracy and precision obtained with six-point and one-point calibration were systematically compared. Finally, the applicability of the described assay was tested by analysis of real samples from brain death cases.

Results: The analytes were fully separated and sensitively detected. No interfering peaks were detected in blank plasma samples from ten different sources. The assay was linear from 0.25 to 10 mg/l for pentobarbital and thiopental, from 0.125 to 10 mg/l for methohexital, from 2.5 to 50 mg/l for phenobarbital, from 0.05 to 2.5 mg/l for diazepam and nordiazepam, and from 0.01 to 0.5 for midazolam. Using six-point calibration, accuracy data (in terms of bias) ranged from -17.9% to 23.7%. Within-day and intermediate precision data (expressed as CV) ranged from 1.4% to 6.4% and from 2.6% to 7.0%, respectively. Using one-point calibration with a calibrator close to the center of the linearity range, accuracy data (in terms of bias) ranged from -11.6% to 29.7%. Within-day and intermediate precision data ranged from 1.3% to 6.2% and from 2.6% to 9.6%, respectively. Recoveries ranged from 85% to 109%. The acceptance criterion defined by the Clinical Toxicology Committee of the GTFCh (99% confidence interval of measured mean within ±50% of target value) was easily fulfilled for all analytes, even with one-point calibration. The assay was successfully applied to analysis of real brain death cases.

Conclusion: The described assay allows rapid, fast and reliable determination of analytes relevant in the diagnosis of brain death. Systematic studies showed that the assay can be performed with one-point calibration. This is an important advantage, because it keeps the workload low for the usually single cases and because results are needed quickly in this context.

Keywords: brain death, GC-MS, validation

NEONATAL ABSTINENCE SYNDROME IN METHADONE-EXPOSED INFANTS IS ALTERED BY LEVEL OF TOBACCO EXPOSURE

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In utero exposure to tobacco has been associated with lower birth weight infants, pre-term births, smaller head circumference, intrauterine growth retardation and increased fetal morbidity; however, few studies have examined the neurobehavioral effects of prenatal tobacco exposure. This report describes the effect of prenatal tobacco exposure on neonatal abstinence syndrome (NAS) for infants born to methadone (mean dose 77.0 mg/day ± 19.4, range 40-110) maintained mothers. Twenty-nine pregnant women and their infants participated in this IRB approved study. Smoking histories were obtained by maternal self-report at enrollment into the study and at delivery. Sixteen women, light smokers (LS), reported cigarette consumption of 10 or less cigarettes/day (mean 8.4 ± 2.3) and thirteen women, heavy smokers (HS), reported smoking 20 or more cigarettes/day (mean 21.5 ± 5.5). The onset, peak and duration of NAS were examined. Infants born to mothers in the HS group had significantly higher ($p=0.014$) NAS peak scores of 9.8 ± 4.8 as compared to 5.6 ± 3.8 as seen in the LS group. There was a significant difference ($p=0.016$) in time to peak between the HS group (113.8 ± 90.0 h) and the LS group (37.8 ± 33.8 h). The duration of NAS showed a trend towards statistical significance ($p=0.054$) between the HS (mean 9.5 ± 7.3 days) and LS (mean 5.1 ± 4.6 days) groups. These data showed that infants born to mothers in the HS group had a 57% higher NAS peak score, took 33% longer to peak and had a 54% increase in NAS duration as compared to the LS group. These results demonstrate the need for future studies to examine the role of tobacco exposure as a variable in the examination of opioid associated NAS.

Keywords: Prenatal, Methadone, Nicotine, NAS, Outcomes

BIOMONITORING OF EXPOSURE TO CHEMICAL WARFARE AGENTS

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Methods to analyze chemical warfare agents (CWA) and their decomposition products in environmental samples were developed over the last decades. On the other hand, methods for such analyses in biological samples have only recently become available. Retrospective detection of exposure to CWA can be useful for various applications. With regard to the "Homeland Defense" program, it can be envisaged that rapid diagnostic methods can play a pivotal role in case of a terrorist attack with CWA. In the same context, confirmation of non-exposure of worried citizens is of utmost importance. Also, such methods can be used for forensic analyses in case of suspected terrorist activities ("chemical fingerprints"). It is self-evident that these methods will also be highly valuable from a military point of view, *e.g.*, to establish firmly to which chemicals casualties have been exposed to, which is a starting point for adequate medical treatment, or for health surveillance of workers in destruction facilities of chemical warfare agents. This presentation will focus on a number of specific methods currently available for verification of exposure to the most common CWA, *i.e.*, nerve agents and mustard agents.

There are basically four methods to diagnose an exposure to a nerve agent:

1. cholinesterase inhibition measurements
2. analysis of hydrolysis products, *e.g.*, alkyl methylphosphonic acids
3. analysis of generated phosphofluoridates after treatment of blood with fluoride ions ("fluoride reactivation")
4. mass spectrometric analysis of phosphylated peptides after enzymatic digestion of modified cholinesterase.

For mustards, there are three distinct methods to assess an exposure:

1. mass spectrometric analysis of low molecular urinary metabolites
2. analysis of DNA adducts by means of mass spectrometric or immunochemical methods.
3. mass spectrometric analysis of protein adducts, *e.g.*, to hemoglobin and albumin.

This presentation will focus on methods that are based on the analysis of long-lived protein adducts, *i.e.*, on methods 3 and 4 for nerve agents and on method 3 for mustards. Advantageously, protein adducts are stable and therefore detectable weeks or even months after the exposure, in contrast to DNA adducts and urinary metabolites which are excreted much more rapidly. The developed technology will be described briefly and examples of real exposure incidents will be presented.

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Keywords: chemical warfare agents, adducts, diagnosis

ATTEMPTED SUICIDE BY INGESTION OF CHLORPYRIFOS: IDENTIFICATION IN SERUM AND GASTRIC CONTENT BY GC-FID/GC-MS

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The case history, toxicological findings, and poisoning characteristics of an attempted suicide by ingestion of a chlorpyrifos formulation are reported along with a description of the validated analytical method. Chlorpyrifos, an organophosphate pesticide introduced by Dow Chemical Company in 1965 as a broadspectrum insecticide, is the active ingredient in many commercial insecticide formulations available in Spain. Organophosphate insecticides inhibit cholinesterase activities which causes accumulation of acetylcholine at synapse, and as a result an overstimulation of neurotransmission occurs. The mortality rate of suicide poisoning is usually high and therefore early diagnosis and appropriate treatment is often life saving. Other compounds can be present in organophosphate formulations and are responsible for part of the toxicity of these commercialized products. In fact, aromatic hydrocarbons, such as toluene and/or xylenes, and other additives could increase the risk of toxicity after ingestion of pesticide formulations. Numerous cases of acute nonfatal and fatal poisoning because of the inhalation or ingestion of chlorpyrifos have been reported in the literature. However, there is a lack of chlorpyrifos poisoning cases published where analytical findings were included.

A 15-year-old female teenager went to the emergency room after the ingestion of a product from a bottle marked with a label "Poison". On admission she was obtunded, with normal vital signs and a strong smell of solvent. Therapeutic measures included the application of decontamination procedures, oxygen and gastric protectors. She had a good outcome with mild CNS depression and bradycardia. Two hours after ingestion biological samples were collected in the emergency room and sent for analysis to our laboratory with instructions to investigate the presence of solvents. The serum and gastric content contained 5.3 and 9.4 μg/mL of unmetabolized chlorpyrifos, 4.6 and 6.9 μg/mL of toluene, and 2.5 and 7.9 μg/mL of butyl acetate, respectively. The toxics were isolated after liquid-liquid extraction of 3 mL of sample with 1 mL of diethyl ether using n-octyl-benzene as internal standard. The simultaneous determination of chlorpyrifos, toluene, and butyl acetate were performed using the combination of gas chromatography with flame ionization detector (GC-FID) for screening analysis, and gas chromatography-mass spectrometry (GC-MS) for confirmation of the obtained results. Both gas chromatographs were equipped with methylsilicone capillary columns. The previous GC-FID screening analysis was used for quantitation of toluene and butyl acetate, and GC-MS SIM mode was used for quantitation of chlorpyrifos, using serum calibration curves in the range of 0.1-5 μg/mL. Limits of detection were 25, 36 and, 23 ng/mL for chlorpyrifos, toluene, and butyl acetate, respectively. Absolute recoveries were more than 90 %, intra-assay precisions less than 5 %, and linearity up to 5.0 μg/mL for all the analytes.

The method provides an excellent and rapid tool for use in cases of unknown poisonings allowing the simultaneous determination of a wide variety of pesticides and additives, including petroleum distillates, in the performance of systematic toxicological analysis in forensic and clinical laboratories.

Keywords: Chlorpyrifos, Poisoning, GC-FID, GC-MS

PROPYLENE GLYCOL IN EXTREMELY HIGH ION GAP LACTIC ACIDOSIS

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A 31-year-old male presented to the ED disoriented in acute distress. His admission laboratory data were remarkable for the degree of acidosis present. An arterial blood gas measurement showed his pH to be 6.85 (reference range 7.35 to 7.45) with a pCO2 of 14 mm Hg (reference 35 to 45) and a lactic acid of 30 mmol/L (reference range 0.7 to 2.1). Additional labs included an ethanol of 217 mg/dL, and a measured osmolality of 376 mOsm/kg (270 – 310). Taking the effect of ethanol into account, the patient had an unexplained osmol gap of 65 mOsm/kg. In addition to the unexplained osmol gap the patient had an anion gap of 50 mEq/L (reference range 10 to 20), the L-lactate of 30 explained this gap. Based on the unexplained osmol gap, the acidosis, and a high anion gap, stat methanol and ethylene glycol were ordered. Negative finding for methanol and ethylene glycol prompted a search for other causes of hyperosmolality. Discussion of the case with the toxicology laboratory revealed the presence of a large peak on the gas chromatographic tracing from a volatiles screen that was not reported initially. This peak represented 40 mg/dL of propylene glycol. Either ethanol or propylene glycol can result in a high anion gap lactate acidosis. The profound acidosis and extremely high L-lactate was a result of the patient drinking for 5 days. While the osmol gap could not be completely explained, the high anion gap could be explained by L-lactate and the implied D-lactate resulting from the propylene glycol metabolism.

Based on the laboratory data, the patient was treated with intravenous bicarbonate and IV fluid replacement in an attempt to correct the acidosis. Despite 4 ampules of bicarbonate, the patients arterial blood gas pH did not increase significantly nor was there a significant increase in urinary output. Due to the impending renal failure and acidosis, the patient was hemodialyzed. Four hours of hemodialysis corrected his acid/base imbalance. Unfortunately, after hemodialysis, the patient developed acute respiratory distress syndrome and required ventilation assistance. The patient also became septic and was treated with antibiotics. After a twelve day hospital stay which included intermittent hemodialysis for renal failure, the patient recovered and was discharged in apparent good health.

This presentation will review the common causes of hyperosmolality in clinical toxicology and the diagnostic pathway for identifying various intoxicants that cause high anion gap metabolic acidosis. The importance of communicating directly with the laboratory to help identify unusual causes of combined osmol/anion gap metabolic acidosis will be emphasized.

Key words: propylene glycol, metabolic acidosis, ethanol

CORRELATION BETWEEN METABOLIC ACIDOSIS AND CLINICAL PARAMETERS IN DIETHYLENE GLYCOL (DEG) POISONING VICTIMS

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This work analyzes fifteen victims of a massive intoxication that took place in Argentina in 1992 as a result of the intake of propolis syrup: a popular medicinal agent used in the 90s for upper respiratory system infections. Diethylene glycol (DEG) was found as the responsible agent which caused metabolic acidosis, anuria, renal failure and death in the 15 studied victims.

DEG poisoning cases were classified into three groups according to the survival time of the victims, namely: 1) those who survived up to 3 days; 2) those who survived from 3 to 5 days; and 3) those who survived from 5 to 21 days.

A methanolic fraction extracted in a Soxhlet system with subsequent concentration and purification was obtained from viscera and blood. Gas Chromatography and FID detector (GC/FID) methodology was performed. On the other hand, samples of the propolis syrup from each of the victims was studied through NMR and quantified by GC/FID, using ethylene glycol as internal standard. Finally, each clinical history from victims were studied in detail: anionic Gap (AG), Excess base (EB) and pH.

Patients belonging to group 1 showed the highest AG values and the lowest (EB) values as well as the major severity in their clinical manifestations. Correlation between pH and EB was r^2 =0.68, 0.99 and 0.55 for groups 1, 2 and 3 respectively. In 3 out of 15 fatal cases studied, DEG could be isolated from viscera and blood. The concentration ratio $(DEG)_{viscera}/ (DEG)_{blood}$ ranged 1.45 – 1.55 with a coefficient correlation of r^2 = 0.96. In the other victims, DEG could not be detected. This could be due to the long survival period of the deceased victims from the syrup ingestion to death, and to the fact that putrefying mechanisms could be operating.

Results showed that the syrup samples contained DEG in a 24% to 65.0% (p/v) range. A proper correlation between the amount of DEG ingested and the anionic Gap (r2=0.63-0.78) could be observed in the victims studied, according to theoretic amount of syrup ingestion (5 –20 ml). So, the lethal dose for humans in this episode was found to vary from 0.019 to 0.174mg DEG/Kg corporal weight. These results could contribute to the understanding of DEG toxicity parameters On the other hand, they could provide data concerning lethal dose in humans.

Keywords: Diethylene Glycol, poisoning, lethal dose

PHARMACEUTICAL IDENTIFICATION AND QUALITY CONTROL TESTING AT A MAJOR MEDICAL CENTER

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Clinical Toxicology laboratories are often presented with the problem of identifying the contents of pharmaceutical products. These preparations come into question in a variety of situations: the preparation is found outside of it's "controlled" environment, the inappropriate or wholly unexpected response of a patient to the administration of the preparation, or suspicions of drug diversion or abuse by a health care provider. Medical institutions generally address the issue of drug abuse by health care workers through pre-employment urine drug testing, random drug testing, and/or "for cause" drug testing after an incident has occurred. However, such programs seldom, if ever address issues of pharmaceutical compounding or tampering.

We have developed a simple HPLC method to routinely identify and quantitate pharmaceutical preparations for over 15 different drugs, including opiates, synthetic opiates, local anesthetics, and midazolam. The method uses a Beckman ODS column (4.6 x 250 mm), and an isocratic mobile phase consisting of acetonitrile: perchloric acid: water (33:0.134:67) for drug separation. Drugs are detected and quantitated using a UV detector at either 206 or 280 nm. In the past 6 years, we have analyzed thousands of pharmaceutical products for content and concentration. Most of these products were analyzed for concentration, to verify proper compounding by our hospital pharmacy or local pharmacies.

Several cases will be presented of typical findings from products that have not been tampered with, as well as, instances of mistaken compounding and drug diversion. Cases include: preparations from patient controlled anesthesia, IV bag solutions, rescue squad drug boxes, and improper compounding of pediatric preparations for clonidine, and hydromorphone.

Keywords: Drug Diversion, Pharmaceutical Testing, Narcotics

METHAMPHETAMINE DETECTION IN URINE OF CHILDREN CO-EXISTING WITH CLANDESTINE METHAMPHETAMINE MANUFACTURING

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In 2000, the Washington State legislature enacted the "Drug Endangered Children's Law" to protect children found at clandestine methamphetamine manufacturing sites. The law mandates that children found at these sites be placed in protective custody and allows for a mandatory two-year enhancement on any "Unlawful Manufacturing of a Controlled Substance" conviction when children are present at the clandestine lab site. In March 2002, the legislature passed a follow-up bill mandating that any person allowing his/her child to be present at a methamphetamine lab, be charged with a felony child endangerment charge, regardless of whether they were charged with the manufacture of methamphetamine. The Washington State Toxicology Laboratory is tasked with providing forensic toxicological services for the coroners and medical examiners and all police agencies within the 39 counties of the state. We began receiving samples (typically urine) as part of the Drug Endangered Child program in 2000, without advance notice and without a suitable protocol for appropriate testing. Over the past two years, we have coordinated with two police agencies to develop a suitable sample collection and testing protocol.

The Washington State Toxicology Laboratory received 26 samples, from February 2002 through February 2004, collected from children for investigation of drug endangerment; 5 diapers, 1 serum and 20 urine samples were submitted to the laboratory for analysis. The ages of the children ranged from 4 months to 16 years of age, mean and media age of 7 years, half of the subjects were female and 23 of the 26 cases (88%) were positive for methamphetamine.

In most of the cases (20 of the 23 positives), the concentrations of methamphetamine and amphetamine in the samples were relatively low, between 0.02 mg/L to 0.2 mg/L methamphetamine. In these cases, it is reasonable to state that the children ingested methamphetamine but may not have exhibited any associated effects. Of the remaining three cases, two exhibited signs of methamphetamine intoxication at the time of their encounter with the police and were treated in the hospital for their symptoms. The first, a 6-month-old male, had a serum methamphetamine concentration of 0.12 mg/L and the second child, a 2-year-old male, had urine concentrations of 13.59 mg/L methamphetamine and 0.9 mg/L amphetamine. The third case with high drug concentrations was a 16-year-old male with urine methamphetamine of 0.05 mg/L and amphetamine of 18.5 mg/L.

Children living in a methamphetamine-manufacturing environment are endangered by many associated factors, the toxicity of the associated chemicals used in the manufacture, inherent fire danger, neglect and are at a higher risk for sexual abuse. The presence of methamphetamine is an indicator that the children have been in unsafe environment and this data can be used to support a case of child endangerment. Child endangerment charges have been filed in at least 12 of the cases; in one case the defendant was convicted of illicit methamphetamine manufacture and he received a sentence enhancement for the child endangerment charge.

Key Words: Methamphetamine, Children, Clandestine

AN EVENT ASSOCIATED WITH FATAL 2,5-DIMETHOXY-4-BROMOAMPHETAMINE OVERDOSE

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2,5-dimethoxy-4-bromoamphetamine (DOB) is a strongly acting hallucinogen with effective dose estimate for an 80 kg man being 2 mg (1). In the event reported here, two men were provided a capsule with white powder containing an unknown substance in order to test its effects as a new LSD like hallucinogen. They shared the content of a capsule and consumed it orally followed with strong rapid hallucinations and vomiting, unconsciousness and coma lasting several days. After unknown time elapsed since the application, they were admitted to the hospital in coma state without response to analgesic irritation. The man AX 28 years (113 kg) survived. The man BX 29 years (65 kg) experienced convulsions, metabolic acidosis (pH 6.6) and died after six days. Immediately after the admission to the hospital, gastric content, blood and urine specimens were sampled and sent for toxicological examination. Alcohol in blood samples was not found. Cedia urine screening indicated the presence of THCOOH in both cases, cocaine and metabolites in AX urine, and no signal corresponding to amphetamines presence was detected. Finally, GC-MS method for unknown drugs discovered the presence of DOB in gastric content and urine samples of both persons. GC-MS targeted analysis for acetylated DOB confirmed its presence in blood of both persons and quantitative analysis provided the concentration values in serum 13ng/ml (AX – survived) and 19 ng/ml (BX – deceased). This report both on nonfatal and fatal DOB overdose cases is based on clear toxicological evidence, moreover it is completed with antemortem DOB blood serum levels and probably it is the first sign of DOB occurence in the Czech Republic.

Reference: (1) A. Shulgin, A. Shulgin: PiHKAL: A Chemical Love Story. Transform Press, Berkeley CA, 1998

Keywords: 2,5-dimethoxy-4-bromoamphetamine, DOB overdose, fatal intoxication

SIMULTANEOUS DETECTION OF STIMULANT LAXATIVES AND DIURETICS IN HUMAN URINE USING GC-MS AFTER ENZYMATIC CLEAVAGE OF CONJUGATES AND EXTRACTIVE METHYLATION

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Background: Laxatives and diuretics are widely abused for various reasons. Surreptitious abuse of both is often associated with eating disorders or Münchhausen syndrome. Concerning laxatives, there is also habitual abuse because of chronic constipation, whereas diuretics are also abused in doping. Such abuse may lead to serious disorders like chronic diarrhea, hypokalemia, dehydration, and disturbance of acid-base balance. Because of the heterogeneity of these side effects and their similarity to symptoms of gastrointestinal or renal disorders, a toxicological screening for laxatives and diuretics should be part of the differential diagnosis of such syndromes. This may also help to avoid extensive and expensive diagnostic work.

Methods: After accelerated enzymatic cleavage of conjugates (glucuronidase/arylsulfatase, EC no. 3.2.1.31/3.1.6.1, 100 000 Fishman units per mL, 50 °C, 90 min), the drugs and their metabolites were isolated from 2 mL of urine by extractive methylation. For details of extractive methylation see Maurer HH et al., JAT 25, 2001, 237. The extract was reconstituted in 50 µL ethyl acetate and 2 µL were injected into the GC-MS (Agilent GC-MSD 5972). Analytes were separated on an HP 1 column (12 m x 0.2 mm I.D.) and detected by mass-spectrometry in the EI full scan mode. They were screened by using reconstructed mass chromatography using selective ions and identified by visual and computerized comparison of the peak underlying mass spectra with the corresponding reference mass spectra (PMW_tox4).

Results: The assay allowed the detection of the diphenylmethane laxatives phenolphthalein, bisacodyl and picosulfate (the latter two via their common metabolites bisacodyl diphenol, methoxy bisacodyl diphenol and dimethoxy bisacodyl diphenol) as well as of anthraquinone laxatives contained in plants like senna, cascara, rhubarb, frangula and aloe via their common metabolite rhein. Furthermore, the diuretics acetazolamide, bemetizide, bendroflumethiazide, bumetanide, butizide, canrenoic acid (also main metabolite of spironolactone), carzenide, chlorothiazide, chlortalidone, clopamide, cyclopenthiazide, cyclothiazide, diclofenamide, etacrynic acid, etozolin, furosemide, hydrochlorothiazide, indapamide, mefruside, metolazone, piretanide, polythiazide, tienilic acid and xipamide could be detected as well as the uricosurics benzbomarone, probenecide and sulfinpyrazone, which are relevant in doping control. Cleavage of conjugates was a prerequisite for sensitive detection of the laxatives and/or their metabolites, because these analytes are excreted mainly as conjugates. For analysis of diuretics, enzymatic hydrolysis is not necessary, because they are mainly excreted unchanged. The limits of detection (LOD, *S/N* 3) of the tested drugs and their metabolites lay in the range of 1 to 500 ng/mL, 90% within 5 to 100 ng/mL. Recoveries were determined for a limited number of analytes (bisacodyl diphenol, butizide, furosemide, hydrochlorothiazide, phenolphthalein, piretanide, probenecide, rhein, sulfinpyrazone, and tienilic acid) representing the different groups of the structurally heterogeneous analytes and ranged from 33 to 99 with coefficients of variation from 4.2 to 21.1 % (determined at 5 x LOD). Applicability studies showed that at least the given drugs and/or their metabolites were detectable over periods of 24 to 72 hours after administration of the lowest therapeutic dose (picosulfate, 5 mg; furosemide, 40 mg; hydrochlorothiazide, 15 mg; spironolactone, 25 mg; or senna plant extract containing 7 mg of sennoside B) to one young healthy volunteer each (after informed consent). Furthermore, the extractive methylation (without the enzymatic cleavage) has proved to be suitable for simultaneous detection of other acidic drugs (Maurer HH, J. Chromatogr. B 733, 1999, 3).

Conclusion: The presented GC-MS procedure allowed simultaneous identification and differentiation of stimulant laxatives, diuretics and/or their metabolites in urine. This procedure should be applicable for diagnosis or differential diagnosis of an abuse of the described drugs or for doping control.

Keywords: Diuretics, Laxatives, GC-MS

DETECTION OF NEW MINOR METABOLITES BY LC/MS AND CHARACTERIZATION BY LC/MS/MS AFTER COMPELLED INGESTION OF COCAINE

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A well-known cocaine addict presented spontaneously to the emergency service of the Lille Hospital (France) and reported that he was forced to ingest a large quantity of a white powder. Surprisingly, the patient did not present any intoxication symptoms but was placed under medical observation. Immunoassay screening of a blood sample did not reveal the presence of any drugs and immunoassays performed on urine led to positive results for cocaine only. LC/MS was used to confirm the immunoassay results and the anticipated metabolites of cocaine were found. In addition, many other minor metabolites were detected and characterized by LC/MS/MS.

Materials and methods: The urine sample was extracted at alkaline pH using a chloroform/isopropyl alcohol mixture (9:1) and evaporated to dryness. 100 µl of mobile phase was added to the extract and 20 µl injected onto the LC/MS system. Chromatographic separation was performed using a Waters Alliance HPLC equipped with a Waters Xterra MS C18 column (150 x 2.1, 3.5 µm). Mass detection was achieved using a Waters ZQ mass spectrometer operated in the electrospray mode in both positive and negative ion polarities. Multi-functional full scan acquisitions were performed at different cone voltages. Well-known urinary metabolites of cocaine were identified through their fragmentation patterns and an additional LC/MS/MS analysis was performed to elucidate the structure of other analytes previously detected in MS mode that could correspond to new non-documented metabolites. Exhaustive LC/MS/MS study (neutral loss, parent and daughters, MRM) was performed using a Quattro Premier (Waters) operated in electrospray combined to modified chromatographic conditions leading to greater retention for the polar compounds and subsequent better separation efficiency.

Results: The LC/MS analysis of the gastric content revealed the presence of cocaine. Subsequent LC/MS analysis of the urine samples led to the detection of many minors metabolites The oral ingestion was confirmed by the positive identification of characteristic metabolites such as m- and p-hydroxybenzoylecgonine and norbenzoylecgonine[1] which has also been found metabolised as N-hydroxynorbenzoylecgonine. It was possible to change the selectivity of the chromatographic separation by using a Xterra column at basic pH (10 mM ammonium bicarbonate) allowing the separation of several new polar metabolites which usually coelute when analysed in acidic conditions.

Conclusion: Thanks to the spontaneous presentation of the patient, the delay between urine sample collection and cocaine oral absorption was very short thus allowing the detection of many minor metabolites of cocaine not usually found because of their very low concentrations. Combining the sensitivity and the selectivity of tandem mass spectrometry with enhanced chromatographic separation, the expected major metabolites were unambiguously identified and many other new metabolites were detected and assigned to compounds such as N-hydroxynorbenzoylecgonine, methoxyecgonine and norecgonine methylester.

References: 1. Klette KL, Poch GK, Czarny R, Lau CO. Simultaneous GC-MS analysis of m- et p-hydroxybenzoylecgonine and norbenzoylecgonine : a secondary method to corroborate cocaine ingestion using nonhydrolytic metabolites. J. Anal toxicol. 2000 Oct ;24(7) : 482-8.

Keywords: Forced ingestion, cocaine, metabolite, mass spectrometry

URINARY EXCRETION RATES OF KETAMINE AND NORKETAMINE FOLLOWING THERAPEUTIC KETAMINE ADMINISTRATION: METHOD AND DETECTION WINDOW CONSIDERATION.

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Ketamine is widely used in veterinary medicine. Its medical application in humans is limited to children because in adults it induces severe psychedelic episodes. In recent years, ketamine has been (ab)used by teenagers as a recreational and club drug because of its hallucinogenic or stimulant effects. Ketamine is also (mis)used as a 'date-rape' drug - to induce amnesia in unsuspecting victims. In a typical scenario, ketamine is surreptitiously added by the perpetrator to the alcoholic beverage of an unsuspecting person, who is subsequently sexually assaulted while under the influence of this substance. Many victims do not report the incident until several days after the event.

This situation creates a demand for sensitive analytical methods to reveal the presence of the drug and/or metabolites in biological specimens collected from the victim. The second very important parameter in drug testing for forensic purposes is the detection window - how long after drug administration a person tests positive for the drug or metabolite.

Sensitive gas chromatography-mass spectrometry negative chemical ionization (NCI-GC-MS) and liquid chromatography-mass spectrometry atmospheric pressure chemical ionization (APCI-LC-MS) methods for the simultaneous quantification of ketamine and its major metabolite - norketamine in urine were developed and validated. These methods were used to study the elimination of ketamine and norketamine in urine collected from six hospitalized children (age 4-13 years) who had received a single intravenous dose of ketamine as an anesthetic for short surgical procedures. The doses ranged from 0.75 to 1.59 mg/kg. Individual urine samples were collected every day or once every two days for 4-16 days.

Target analytes were isolated from urine samples after enzymatic hydrolysis (with ß-glucuronidaze) followed by solid phase extraction (HCX column). Ketamine-D_4 and norketamine-D_4 were used as internal standards. For NCI-GC-MS procedure, extracts were derivatized with HFBA. The monitored negative ions for ketamine derivative were (m/z) 226 and 357, for norketamine, 383 and 399, and for norketamine-D_4, 387 and 403. APCI-LC-MS analyses were carried out without analytes derivatization. Pseudomolecular ions of (m/z) 224 and 228 (for norketamine-D_0 and -D_4), 238 and 242 (for ketamine-D_0 and -D_4) were monitored.

The NCI-GC-MS assay had an LOQ of 20 ng/mL for ketamine and of 50 pg/mL for norketamine, and displayed LOL across a concentration range of 20-1000 ng/mL and 50-1500 pg/mL for parent drug and metabolite respectively. LOQ and LOL for the APCI-LC-MS method were 2 ng/ml and 2-2000 ng/mL for both compounds. For the NCI-GC-MS, mean inter-day precision for ketamine and norketamine ranged from 21.4-30.4% and 16.8-23.6%, respectively. For the APCI-LC-MS, mean inter-day precision for both compounds were between 1.6-3.7%.

Using NCI-GC-MS, ketamine was detected in urine of four persons up to 1 day and in one up to 2 days after drug administration. Its concentrations ranged from 58 to 1181 ng/mL. Norketamine (measured in concentrations of 1.18 µg/mL-50 pg/mL) was detected up to 14 days (average 7 days). Using the APCI-LC-MS method, ketamine was detected in two persons up to 2 days, in one up to 4 days, in one up to 11 days and in one only up to one day at concentrations of 2-813 ng/mL. Norketamine (at concentrations of 1276-2 ng/mL) was detected up to 3, 4, 5 and 6 days after drug administration. In one person, ketamine was not detected through the entire 16-day period using both methods.

Detection window of the analytes is highly dependent on the method used for determination and interindividual variability.

Keywords: ketamine, norketamine, detection window, drug facilitated sexual assault

NOVEL ASPECTS OF IN VITRO METABOLISM OF BUPRENORPHINE

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Buprenorphine is mainly metabolized by cytochrome P450 (P450) 3A4 mediated N-dealkylation to norbuprenorphine; both buprenorphine and norbuprenorphine conjugate with glucuronic acid. Previously, tentative 6-O-desmethyl norbuprenorphine in free and conjugated form and some unknown polar metabolites were observed in rats; while no other metabolites were observed in human. We find higher buprenorphine elimination compared to norbuprenorphine formation in human liver microsomes (HLM), which supports the hypothesis that other metabolic pathways might exist. In the current study, the phase I and phase II metabolism of buprenorphine was investigated in HLM and cDNA-expressed P450s. Metabolites were screened by LC-MS/MS, coupled with precursor-ion scan, product-ion scan and neutral-loss scan.

The incubation for phase I metabolism was performed in a mixture of 0.1 M phosphate buffer (pH 7.4, 1.0 mM EDTA and 5.0 mM $MgCl_2$); the NADPH generating system (10 mM glucose-6-phosphate, 1.2 mM NADP, and 1.2 units gluscose-6-phosphate); and 0.5 mg/ml microsomal protein. The reaction was initiated by the addition of NADPH generating system in a 37°C shaking water bath and continued for 30 min. The incubations of buprenorphine in cDNA- expressed P450s were performed as described above except that 25 pmol cDNA-expressed P450s was used and the incubation time was 20 min. The incubation for phase II metabolism was conducted in the presence of 25 μg/ml alamethicin and 2 mM UDP-glucuronosyltransferases. LC-MS/MS utilized a Finnigan TSQ 7000 equipped with a triple-quadrupole mass spectrometer and an electrospray ionization source.

During phase I metabolism of buprenorphine, 5 metabolites (M1-M5) in addition to norbuprenorphine were identified. M1 and M2 are hydroxylations of buprenorphine at the tert-butyl group and ring moiety, respectively. M3-M5 are secondary hydroxylation metabolites of norbuprenorphine. The hydroxyl groups are on the tert-butyl group (M3) and ring moiety (M4 and M5), respectively. The metabolism of buprenorphine in cDNA-expressed P450s showed that P450 3A5 had highest catalytic activity in M1 formation, with the involvement of 2C8 and 3A7, but their activity is only 5.9% and 3.3% of 3A5, respectively; P450 3A4 has the highest catalytic activity in M3 formation, with contributions of 3A7, 3A5 and 1A2 to a lesser extent, approximately 12.5%, 2.7% and 2.4% of 3A4, respectively. No detectable signal was observed for M2, M4 and M5 by selected reaction monitoring on LC-MS/MS. M3 is the major metabolite of norbuprenorphine in HLM, which is mediated mainly by P450 3A4, with the contribution of 3A5 and 3A7 no more than 6% of 3A4. Norbuprenorphine metabolism suggested that secondary metabolite M3 of buprenorphine was formed through norbuprenorphine. The primary study on phase II metabolism of buprenorphine showed the presence of conjugated hydroxyl buprenorphine and hydroxyl norbuprenorphine, the structural identification will be processed in details in the future work.

Keywords: Buprenorphine, Metabolism, P450

CHANNELING THE EMPEROR; WHAT REALLY KILLED NAPOLEON?

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Those with an opinion about the cause of Napoleon's death rely largely on the results of hair testing. Over 30 different samples, of reasonable provenance, have been analyzed. Some samples were obtained during Napoleon's first exile in Elba, years before he arrived on Saint Helena. Others were obtained at autopsy. All studies have revealed elevated concentrations of arsenic and, in the most recent study, elevated concentrations of antimony as well. These observations, coupled with written accounts of the symptoms and signs exhibited by the Emperor, have led to near universal agreement that arsenic poisoning was the cause of death. For proponents of this view, the only question remaining is whether poisoning was accidental or intentional. Evidence for intentional poisoning comes from the Emperor's own hand. He wrote in his will, "I die before my time, murdered by the British oligarchy and its hired assassin." Others maintain that exposure was environmental. Many sources of environmental contamination have been proposed, but there is compelling evidence that Napoleon was poisoned by his own wallpaper; it was painted with "Scheele's green" pigment, a mixture of copper arsenides. It has been known for more than 100 years that certain molds, some likely to have been present in Napoleon's wall coverings, can volatilize arsenical salts. Other explanations are possible. Those favoring the poisoning theory tend to minimize the facts that Napoleon had a rather thorough autopsy. He had requested one because he believed that he, like his father, might have stomach cancer, and that his children were at risk. The autopsy was performed by one of Napoleon's personal physicians, a Corsican named Francesco Antommarchi. Five English physicians were present, but since Antommarchi was the only one actually trained in anatomic pathology, he performed the dissection. Though some physicians later expressed divergent opinions about the cause of death, none disputed his anatomic findings: an ulcerated, regionally invasive, carcinoma of the greater curvature, metastasizing into the regional lymph nodes. Since high levels of arsenic were clearly present at death and, according to the latest studies, present for many years, the question to be answered is whether Napoleon died "of" arsenic poisoning, or "with" arsenic poisoning. Recent developments in the treatment of promyelocytic leukemia (APL) may provide the answer. Treatment with arsenic trioxide is complicated by the occurrence of QT prolongation, torsades des pointes, and sudden death. Tissue culture studies have shown that at clinically relevant concentrations, arsenic blocks both I_{Kr} and I_{ks} channels and, at the same time, also activates $I_{K\text{-}ATP}$ channels. The effects cancel each other out, and normal cardiac repolarization is maintained. The unpredictability of QT interval prolongation, and the occurrence of ventricular arrhythmias during arsenic therapy in APL patients, is the result of competing effects, blocking and activating multiple repolarizing potassium currents. The balancing of these forces is clearly a delicate matter, one that could be disrupted by any number of extrinsic or intrinsic physiologic forces. Napoleon was given a huge dose of tartar emetic (potassium antimony tartrate) the night before he died. Antimony binds to potassium channels. It appears that the immediate cause of death was neither gastric carcinoma, nor chronic arsenic poisoning, but medical misadventure. Had Napoleon not been given the tartar emetic, arsenical effects on cardiac conduction would have remained balanced; he would have lived to die a natural death, probably from gastric carcinoma.

Key Words: Arsenic, Napoleon, Channelopathy

Scientific Session Abstracts:

Forensic Urine Drug Testing & Adulteration

COMPARISON OF SIMULATANEOUS CAMP AND BMBP MULTIPLE ANALYTE ENZYME IMMUNOASSAY REAGENTS WITH ABBOTT AND SYVA EMIT SINGLE ANALYTE IMMUNOASSAY REAGENT

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The development of drugs of abuse testing (DOA) has seen significant changes since the principals competitive binding of Yalow and Solomon were adapted to DOA screening. The changes in the governing regulations, the instrumentation and testing requirements has led to more emphasis being placed on turn around times and cost of the test to the client. The many advances in antibody production have enhanced the selectivity and specificity of screening tools much to the advantage of the laboratory and the client.

This investigator has begun the evaluation of a Multiple Analyte Enzyme Immunoassay (MAEI) products: specifically, 1.) Cocaine-Amphetamine-Morphine-Phencyclidine (CAMP) and 2.) Barbiturate-Methadone-Benzodiazepine-Propoxyphene (BMBP) recently approved by the FDA for use in the U.S. The design of the dual reagent system CAMP and BMBP takes into account the values that are associated with the widely accepted NIDA/ SAMHSA screening cut-offs. The reagents are designed to test the four (4) drugs in each group in a single sampling. The resulting response of the instrumentation will be relative to a previously determined cut-off value. Any result being above the threshold for a positive will be subjected to individual analyte analysis. The samples giving no response will be considered negative and no further testing would be performed. The reagent design is based on certain statistical data, specifically, > 90% of all urine drug screens are negative. The design of the reagent system will enhance the turnaround time and costs associated with testing operations while still holding the same high quality standards required of the testing facilities.

This report will focus on the comparison of the MAEI reagents with previously tested samples. The discussions will include comparisons of the MAEI with several reagent systems including, but not limited to, Abbott (FPIA), and SYVA EMIT. The correlations of these analysis and discussion of the reagent system will be presented. The preliminary testing has been completed and will be furthered after the submission of this abstract to include statistics, cross reactivity and overall effectiveness in the testing for drugs of abuse.

Keywords: Multiple Analyte Enzyme Immunoassay, drugs of abuse, turnaround time

URINE ADULTERATION TRENDS FROM 2001-2003

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The use of urine adulterant products to defeat a urine drug test has become an increasingly important issue for the federal and private drug testing programs. Initially, adulteration products were common household chemicals. In approximately 1990, the first of designed adulterants appeared as Urine Luck containing glutaraldehyde. Nitrite quickly followed once the detection of glutaraldehyde was established. Since that time a number of products have evolved with chromium VI, iodine, iodate, and fluoride as the primary purchased adulterants. And another disturbing trend in the increase in the number of substituted and invalid specimens reported by the laboratory. These specimens indicate the increased use of excessive hydration to lower urine levels of drugs of abuse or in the case of invalid specimens, the use of an unidentified adulterant.

The analysis of adulteration trends includes only specimens tested by Northwest Toxicology during the period of 2001-2003. The total number of specimens during each year was approximately 600,000 samples per year. Each specimen tested by the laboratory receives an analysis for creatinine (specific gravity if creatinine is less than 20 mg/dL), pH, and adulterants. During 2001-2002, the adulterant test was an analysis for nitrite and chromium. In 2003, the nitrite and chromium tests were replaced by a general oxidant test. The analysis of the data for the three year period reveals several significant trends in specimen validity testing (SVT). The number of specimens reported as substituted or invalid has more than doubled from 2001 to 2003 while chromiumVI positive specimens have been reduced by 75%. Nitrite positive specimens have been reduced by 20% with unexpected increases in bleach and abnormal pH specimens. Iodine and iodate analysis began in 2002 and has quickly become the number one detected adulterant by the laboratory. Fluoride is frequently encountered when iodate is also present.

	2001	2002	2003
Substituted	136	192	248
Unsuit/Inv	202	248	442
Nitrites	98	88	77
Chromium	241	102	58
Bleach	5	30	20
Soap	1	3	1
pH	48	72	97
Iodine/Iodate	NA	30	105
Fluoride	NA	NA	9
Glutaraldehyde	0	0	1
	0.12%	0.13%	0.16%

The number of adulterated specimens clearly indicates that the routine testing for a single adulterant, such as nitrite, will not detect the majority of adulterated samples and that a comprehensive SVT program must be developed for the identification of these specimens.

Keywords: Specimen validity testing, adulteration, nitrite, chromium

ANALYSIS OF URINARY STEROID SULFATE METABOLITES USING ION-PAIRED EXTRACTION

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Urinary steroid metabolites may be excreted in their unchanged (free) form, and/or more likely, as glucuronide or sulfate conjugates. Routinely, doping control laboratories accredited by the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA) screen urine samples collected from athletes for the presence of free and glucuronide conjugated steroids by Gas Chromatography-Mass Spectrometry (GC-MS) following enzymatic hydrolysis and solid phase extraction. There is a clear need, however, for laboratories to analyse a number of steroids excreted primarily as their sulfate conjugates in order to further metabolic research and develop confirmation methods that are more specific to particular steroid administrations. Published methods utilising a range of chemical hydrolysis techniques to cleave the sulfate moiety from steroid metabolites and make them amenable to GC-MS analysis have been problematic to implement due to urinary matrix effects. To circumvent this problem, ion-pairing extraction with (-)-N,N-dimethylephedrinium bromide under basic conditions was optimised to obtain a purified sulfate fraction. Cleavage of the sulfate moiety was efficiently achieved by chemical hydrolysis under mild conditions using methanolic-HCl derived from trimethylchlorosilane (TMCS). The free steroids were extracted into hexane at pH 9.8 before reaction with N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) to form TMS enol ether derivatives that were subsequently analysed by GC-MS. This method provided linear recoveries ($R^2 \geq 0.9993$, 100 to 5000 ng/mL) of greater than 90% for androsterone (5α-androstane-3α-ol-17-one) extracted as the sulfate conjugate. The potential of direct urinary sulfate metabolite analysis using Liquid Chromatography-Mass Spectrometry (LC-MS) is also discussed as a means to alleviate the need for chemical hydrolysis. While these methods are directed at steroid detection for IOC/WADA laboratories they may be applied in a broader toxicological sense for the analysis of sulfated metabolites of other drugs of abuse. Novel analysis methods such as these may also allow more detailed metabolic studies to be carried out to determine new markers of specific drug abuse, thus demonstrating their potential as a valuable confirmation technique. Incorporated into the GC-MS method validation was an evaluation of a reasonable estimate of measurement uncertainty that is presented as an example to emphasise the importance of ISO 17025 compliance for forensic toxicology laboratories.

Keywords: urinary sulfate metabolites, ion-paired extraction, measurement uncertainty

PAPAIN, A NOVEL URINE ADULTERANT

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The estimated number of employees in the United States screened annually for illicit drugs is approximately 20 million, with marijuana being the most frequently abused drug. Urine adulterants provide an opportunity for illicit drug users to obtain a false negative result on commonly used primary drug screening methods such as the Fluorescence Polarized Immunoassay (FPIA) technique. Typical chemical adulterants such as nitrites are easily detected or render the urine specimen invalid as defined in the proposed SAMHSA guidelines for specimen validity testing based on creatinine, specific gravity and pH.

Papain is a cysteine protease with intrinsic ester hydrolysis capability and several residues that serve as hydrophilic and hydrophobic binding sites that can act as a potential urine adulterant. These mechanisms would exists in a novel class of urine adulterants and urine adulteration with hydrolytic enzymes can be attained with a relatively smaller quantity as compared to their typical chemical counterparts. The primary metabolite of the psychoactive chemical in marijuana, 11-norcarboxy-delta-9-tetrahydrocannibinol (11NC), was assayed by FPIA in concentrations ranging from 25 to 500 ng/mL, at pH values ranging from 4.5 to 8, over the course of 3 days with papain concentrations ranging from 0 to 10 mg/mL. FPIA analysis of other frequently abused drugs: amphetamines, barbiturates, benzodiazepines, cocaine, opiates, and phencyclidine, along with gas chromatography / mass spectrometry (GC/MS) of 11NC and high performance liquid chromatography / ultraviolet (HPLC/UV) of nordiazepam was performed in order to determine if the mechanism of urine adulteration by papain was analyte specific. Control and adulterated urine specimens (n=30) were assayed for creatinine, specific gravity, osmolarity and pH to determine if papain rendered the specimens invalid based on the proposed SAMHSA guidelines. There was a direct pH, temperature, and time dependent correlate between the increase in papain concentration and the decrease in 11NC concentration from the untreated control groups ($p<0.01$). The average 72-hour 11NC concentration decrease at pH 6.2 with a papain concentration of 10 mg/mL was 50%. GC/MS of 11NC revealed a 67% decrease in concentration with a 24-hour incubation at room temperature (22 °C) and a papain concentration of 10 mg/mL. Papain did not significantly decrease the concentration of the other drugs analyzed with the exception of nordiazepam. None of the specimens were rendered invalid by the parameters of specimen validity testing with the addition of a maximum concentration of 10 mg/mL papain. Twice recrysatllized papain (1mg/mL) that was deactivated by E-64 yielded a 33% decrease in the reported value of 11NC by FPIA analysis. The mechanism of interference with FPIA analysis is analyte specific for 11NC and nordiazepam and does not require active enzyme, which does not allow detection of papain by a rapid enzyme activity assay. Immediate FPIA analysis and/or refrigeration is suggested to minimize the interfering effects of papain with regards to 11NC analysis.

Key words: 11-nor-delta-9-carboxy-tetrahydrocannabinol, immunoassay, protease

IODINE CONTAINING ADULTERANT – ITS EFFECT ON RAPID DRUG SCREEN AND DETECTION BY INTECT® 8

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Aims: New generation of commercial adulterants have included iodine as the main ingredient at sample concentration of about 1 mg per ml. The objective of this study was to evaluate iodine's effect on a certain rapid drug screen and its detection by an onsite adulterant dipstick, Intect®8.

Method: The adulteration effect of iodine was studied by adding iodine to a urine sample containing three times the SAMHSA cut-off concentrations of five drugs including benzoyl ecgonine (COC), morphine (OPI), amphetamine (AMP), THC and PCP. The final concentration of iodine in the sample was 1 mg/ml. The adulterated sample was then tested by an onsite rapid drug screen Monitect® PC11 over one hour period. The same sample was also tested with the new Intect 8 onsite adulteration test strip that contains 8 test pads including creatinine (CR), nitrite (NI), glutaraldehye (GL), pH, specific gravity (SG), bleach (BL), pyridinium chlorochromate (PCC) and iodine (I) to evaluate the adulterant's detection over time.

Results: Negative (-) result in the drug screen indicates adulterant was able to modify the positive drug test result to negative result whereas (+/-) indicates a possible adulteration effect for that specific drug; normal (N) result in the Intect 8 pad suggests no effect of iodine on the specific test pad while abnormal (A) indicates detection of the adulterant by the specific pad.

	Time (min.)	COC	OPI	AMP	THC	PCP
Drug Screen Result	5	Invalid results due to no control line				
	10	+	+	+	-	+
	30	+	+	+	-	+
	60	+	+/-	+	-	+

	Time	CR	NI	GL	pH	SG	BL	PCC	I
Intect 8 Result	5	N	N	N	N	N	A	A	A
	10	N	N	N	N	N	A	A	A
	30	N	N	N	N	N	A	A	A
	60	N	N	N	N	N	N	N	N

Urine spiked with PCC or BL did not cause an abnormal result with the Iodine pad.

Conclusion: Iodine containing adulterant is shown to mask the presence of THC. It also has some effect on opiate. This adulterant is detectable by an onsite adulteration test device Intect®8 in a 30-minute window.

Keywords: Adulteration, Intect, iodine

A COMPARATIVE EVALUATION OF THE INSTANT-VIEW 5 PANEL TEST CARD WITH ONTRAK TESTCUP PRO 5: COMPARISON TO GC/MS AND INSTRUMENT SCREENING WITH ONLINE REAGENTS

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We have compared the ability of two on-site testing devices, Instant-View Test Card (Alfa Scientific Designs, Inc., Poway, CA) (I) and OnTrak TesTcup (Roche Diagnostics Corp., Indianapolis, IN) (T) to discriminate negative from positive urine samples for the following five drug groups: cannabinoids, cocaine metabolite, opiates, amphetamines and benzodiazepines. Samples for a precision study were prepared for each device separately due to cutoff and primary antigen differences. A drug-free urine pool was fortified in a random fashion to contain the primary antigen at seven concentrations (0, 25, 50, 75, 125, 150 and 175% of cutoff). These stocks were than submitted for GC/MS confirmation and then 10 aliquots from each where prepared and assigned random numbers of 1-70 for each test device. All drug groups, except for cannabinoids were within 10% of target and target concentrations were used for the evaluation. Cannabinoids deviated more than 15% from target. The GC/MS-determined concentrations were used for cannabinoids; this resulted in samples at 0, 62, 80, 102, 136, 158 and 170% of cutoff. Two individuals tested each sample. None of the 0 or 25% (0 or 62% for cannabinoids) cutoffs tested positive with any device. The results for the rest of the samples can be summarized as follows where for each device we first list the % of below cutoff samples testing positive and the % of above cutoff samples testing negative: cannabinoids (I-3.3, 47.5; T-0, 40); benzoylecgonine (I-25, 8.3; T-16.4, 0); morphine (I-2.5; 21.7; T-10, 0); amphetamines (I-0, 60; T-22.5, 0) and benzodiazepines (I-8.8, 15; T-0, 21.7). Similar data were collected for an automated immunoassay using OnLine reagents; the only imprecise result was for cannabinoids at 102% of cutoff where 15% of the samples tested negative. Samples submitted to NWT were used for the clinical study. Seventy-five samples that recently screened negative were rescreened for the five drug groups and the fifty with the lowest absorbance readings were selected as negative samples. Positive samples were selected from actual positives that had reached their disposal date. They were rescreened for all five drug groups. Those with results > the 75% control in the screening batch were then submitted to GC/MS confirmation for the particular drug group. Sufficient samples were screened and confirmed to get at least 45 samples above the device specific cutoff; with many samples near cutoff and some just below cutoff. As each on-site device will test for all five drug groups simultaneously, this provided ≈ 150 additional samples that were presumptively negative per drug group. Samples were assigned random numbers, and two individuals tested each sample. Providing results as the percent of device positives when the GC/MS results (sum of analytes in many case) are <, or ≥ the device cutoff, respectively were as follows: amphetamines (I-0, 76.1; T-6.1, 81.8), benzoylecgonine (I-0, 100; T-0, 100); benzodiazepines (I-4.1, 100; T-5.1, 94.3); cannabinoids (I-1.0, 68.1; T-2.3, 79.1); and opiates (I-0.8, 96.9; T-0, 95.9). In summary, the Instant-View Test Card was less precise than the TesTcup at or near the cutoff; with clinical samples there was, however, an overall similarity. There were more false positive amphetamines for the TesTcup and more false negative cannabinoids with the Instant-View test card. (Supported by NIDA contract N01DA-3-8829)

Keywords: On-site testing devices; immunoassay comparison to GC/MS; urinalysis

Direct Analysis of Opiates in Urine by Liquid Chromatography/Mass Spectrometry/ Mass Spectrometry

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A method for the direct analysis of ten opiate compounds in urine was developed using Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS). Opiates included were: morphine-3-β-glucuronide, morphine-6-β-glucuronide, morphine, oxymorphone, hydromorphone, norcodeine, codeine, oxycodone, 6-monoacetylmorphine (6MAM) and hydrocodone. Deuterated internal standards used were morphine-d_3 and 6MAM-d_6. Urine samples were prepared by addition of the internal standard solution, centrifugation to remove large particles and direct injection into the LC/MS/MS. Gradient reverse phase separation was accomplished on a phenyl column using acetonitrile and water modified with formic acid and ammonium formate. An electrospray ionization (ESI) interface was used to introduce the LC eluent into the MS. Multiple Reaction Monitoring (MRM) was utilized for detection in the MS/MS mode based upon pre-established precursor: product m/z transitions. Separation and detection of all compounds was accomplished within six minutes. Selectivity and possible ion suppression were evaluated using 10 different blank urines. Linearity was established for all opiates except 6MAM from 50 ng/mL to 10,000 ng/mL; 6MAM from 0.25 ng/mL to 50 ng/mL. Correlation coefficients (r) for all opiates was > 0.99. Inter-run precision (%CV) ranged from 1.1 % to 16.7%. Intra-run precision ranged from 1.3% to 16.3%. Accuracy (% Bias) ranged from –7.3% to 13.6% and –8.5% to 11.8 for inter and intra-run respectively. 89 urine samples previously analyzed by GC/MS were re-analyzed by the LC/MS/MS method. The qualitative results found an 88% agreement for negative samples between the two methods and 94% for positive samples. The LC/MS/MS method identified 19 samples with additional opiates in the positive samples. Ten samples were oxymorphone positive that the GC/MS method was not setup to detect. Of these, 90% were associated with GC/MS positive oxycodone samples, which metabolically are justified. Additionally three samples were hydromorphone positive for GC/MS hydrocodone positive samples, which also is likely. The remaining positive samples were oxymorphone, oxycodone, hydrocodone and hydromorphone. Quantitatively it was difficult to compare the two methods as the GC/MS method utilized a glucuronidase step that was not included in the LC/MS/MS method. Therefore only the morphine results could be compared if the LC/MS/MS results for the glucuronide metabolites were summed on a molar basis with the morphine. The R^2 was 0.78. The difference between the methods is probably due to the efficiency of the GC/MS glucuronidase procedure. Overall the direct injection LC/MS/MS method performed well and permitted the rapid analysis (< 6.0 minutes) of urine samples without extensive sample preparation for several opiates simultaneously. Additionally the method provided a lower limit of quantitation for 6MAM than previously reported utilizing direct injection techniques. The method could be further improved with the addition of compound specific deuterated internal standards and other glucuronide metabolites, i.e., hydromorphone.

Keywords: Opiates, LC/MS/MS, Urine

UNUSUAL DRUG TEST RESULTS FROM KNOWN HEROIN USERS

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The use of total morphine levels in urine has traditionally been one approach to predicting the use of heroin. Recently we have encountered several situations that would suggest this practice should be re-visited. Five individuals that were either on supervised release or on parole were tested for illicit drugs as a condition of their release. These individuals were comprised of two black males, one black female, one white male and one white female. The ages of these individuals ranged from 29 to 70 years, with an average age of 51 years. All individuals were experienced with heroin (either use or possession). Based on their history of drug use, these individuals were subject to periodic urine drug testing. These samples were screened for illicit drugs, including opiates, using reagents manufactured by Diagnostic Reagents, Inc. The screening cutoff level for opiates was 300 ng/ml. All samples screened negative for opiates. Due to their past experience with heroin, a special test for 6-AM was requested on each sample. All samples were positive for 6-AM with the concentrations ranging from 10 to 144 ng/ml. To investigate this apparent false negative immunoassay screen, all samples were analyzed for the presence of codeine, total morphine and heroin. All samples were negative for both codeine and heroin. All total morphine levels were less than 300 ng/ml, which substantiated the negative immunoassay results. Total morphine levels ranged from 18 to 245 ng/ml. These results suggest that the traditional approach for predicting heroin use based on total morphine levels need to be looked at more closely, as current practice may in fact underestimate instances of heroin use.

Keywords: heroin, 6-acetylmorphine, morphine

ESTIMATE OF DETECTION PERIOD IN URINE FOR MARKERS OF STREET HEROIN

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There is growing international interest in prescribed pharmaceutical heroin as treatment for individuals who are unable to stop injecting opiate drugs. The rationale for this treatment is one of "harm minimisation". In a previous paper (1) co-workers reported on the GC-MS analysis of over 1000 urine samples from patients attending a substance misuse service during which metabolites of various contaminants of street heroin were detected. They concluded that the detection of papaverine and noscapine metabolites was likely to provide a reasonably sensitive and highly specific means of identifying the illicit use of heroin in patients prescribed diamorphine. We believe that these markers offer the best means of determining the use of street heroin in these patients. The aim of this study was to estimate the detection period for identifying markers in the urine of street heroin users. Over 50 urine samples were collected from patients who admitted to the use of street heroin within the previous 72 hours. The researcher interviewed each subject regarding all episodes of drug use within the preceding 72 hours, including details of amount used, route of administration and time of use. The time the urine was passed was also noted. These samples were analysed by the method currently in use for the routine analysis of urine samples from patients attending drug treatment centres, that is mixed-mode solid-phase extraction (SPE) followed by GC-MS (2). Urine samples were subjected to enzymatic hydrolysis followed by SPE using Bakerbond narc-2 columns. The eluent was selectively derivatised with N-methyl-bis-trifluoroacetamide and N-methyl-N-trimethylsilyltrifluoro-acetamide + 1% trimethylchlorosilane. Analysis was performed using a GC-MS system operating in full scan mode. The study samples were analysed for the commonly abused drugs plus a range of other drugs including antidepressants and certain antipsychotics that are prescribed to these patients. In addition, the metabolites of papaverine, hydroxy and dihydroxypapaverine, and the metabolites of noscapine, meconine and hydroxymeconine, were looked for in each sample. Other contaminants of street heroin including papaverine, noscapine, thebaine, and acetylcodeine were also monitored. Using this assay the limit of detection for morphine was 0.05 ug/mL. The limit of detection for hydroxy and dihydroxypapaverine could not be determined as pure standards were not commercially available. A characteristic peak pattern in the chromatogram was found to be indicative of the presence of the papaverine metabolites, which were then matched by retention time and spectra obtained from the elucidation of these metabolite derivatives as reported previously (1). Initial analysis of the data shows that hydroxy and dihydroxpapaverine were detected in urine for approximately the same period as morphine. This data supports the findings of McLachlan-Troup et al that hydroxy and dihydroxypapaverine can be used to determine if patients prescribed injectable diamorphine are "topping-up" with street heroin. Papaverine, noscapine, thebaine and acetyl codeine were not detected in any of the study samples.

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Keywords: Street heroin markers, papaverine metabolites, noscapine metabolites

EFFECTIVENESS OF FREE AND TOTAL MORPHINE CONCENTRATION AS CRITERIA FOR SELECTING URINE SPECIMENS FOR TESTING 6-ACETYLMORPHINE

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The U.S. Department of Health and Human Services' workplace urine drug testing program has adopted a 2000 ng/mL total morphine concentration as the criterion for testing 6-acetylmorphine (6-AM). We were interested in evaluating whether such a criteria can be applied to specimens collected from workers in Taiwan, a genetically different group than the typical US worker. Two hundred and thirteen workplace specimens, testing positive for opiates by immunoassay (cutoff 300 ng/mL), were analyzed for 6-AM, and for free and total morphine, by GC-MS methods. Recovery efficiencies of extraction and hydrolysis protocols (for total morphine determination) were evaluated and used to derive analyte concentrations in test specimens. In reference to an earlier report by Paul et al [1], data hereby obtained were used to determine whether free or total morphine concentration is more effective in predicting the presence of 6-AM in 2 different concentration ranges: >10 ng/mL and 5–10 ng/mL. As shown in Table 1, 4 specimens were found to contain from 5-10 ng/mL of 6-AM, while 55 specimens were ≥10 ng/mL. This total of 59 specimens showed the presence of 6-AM at >5ng/ml, regardless of whether total morphine (2000 ng/mL) or free morphine (50, 100, or 200 ng/mL) concentration was used as the cutoff for selecting specimens for 6-AM determination.

Table 1. Effectiveness of free and total morphine in predicting the presence of 6-AM

Analyte concn (ng/mL)	No. of specimen	No. of specimen with 6-AM concn (ng/mL) >10	5.0–10	1.0–4.9	Negative^
Total morphine					
≥300	213	55 (25.8%)†	4	14	140
≥2000	162	55 (34.0%)	4	12	91
≥4000	142	55 (38.7%)	3	9	75
Free morphine					
≥50	181	55 (30.4%)	4	12	110
≥100	168	55 (32.7%)	4	12	97
≥200	154	55 (35.7%)	4	11	84

^ Limit of detection: 1 ng/mL.

† Numbers inside parentheses are the percentages of the morphine positive (using the cutoff listed in the first column in respective rows) specimens that were found to contain ≥10 ng/mL 6-AM.

Data resulting from this study indicate comparable effectiveness in using either 2000 ng/mL total morphine or 100 ng/mL free morphine as the criterion for selecting specimens to further test for the presence of 6-AM at the 10 or 5 ng/mL level. The free morphine option is less costly, avoids uncertainty associated with the hydrolysis process, and may produce more nearly accurate results. This study provides an addition to the free morphine-related database and adds further confidence to the use of free morphine data, which is not readily available.

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Keywords: Heroin, free-Morphine, 6-mono-acetyl Morphine

REVIEW OF AN UNUSUAL MULTI-DRUG POSITIVE USAF MEMBER CASE

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The Air Force Drug Testing Laboratory (AFDTL) tests approximately 400,000 military specimens/year for THC, cocaine, amphetamines, PCP, opiates, barbiturates and LSD. Only 88-90 member specimens are positive for multiple drugs. A case report on specimens positive for multiple drugs may have a complex interpretation. The member underwent on consent urinalysis on late Friday evening/Saturday morning. The week preceding this positive result, the individual member had an occupational accident where the cleaner " Googone " was sprayed into his eyes. He was prescribed Tylox for pain resulting from ocular damage.

On the night preceding the drug test, a call was made to 911 after his girlfriend found him unresponsive. EMTs noted dilated pupils and that he was not cooperative. He was transported exhibiting combative and belligerent behavior to a local hospital. At the hospital, the member responded to questions but had lapses in memory. A drug and alcohol screen was performed at the hospital. The alcohol screen was negative but the drug screen was positive for cocaine and THC as benzoylecognine and 11-nor carboxy THC. A consent urinalysis was obtained and analyzed at the Armed Forces Institute of Pathology (AFIP). Analysis for THC, cocaine, amphetamines, PCP, opiates and barbiturates were performed by Axsym screen and GCMS confirmation. Medical history indicated that he had ingested psilocin mushrooms so an analysis for psilocyn was accomplished using a gas chromatography (GC) screen and GCMS confirmation. The following drugs were detected and quantified:

1) Benzoylecognine	3148 ng/ml
2) MDMA	5947 ng/ml
3) Psilocin	6450 ng/ml
4) 11- nor carboxy THC	135 ng/ml

The defense asserted that the individual was not capable of giving consent due to the number of drugs detected and the fact that he was taking Tylox and drinking heavily. There were 15 pills missing. His girlfriend reported that he was dinking beer and mixed drinks. Additional analysis at AFIP revealed the presence of 165 ng/ml urinary oxycodone. Psychiatric analysis was performed for competency to consent. At trial, the military judge ruled the member mentally capable. Investigation revealed that the member the evening of the hospital admission consumed 4-5 caps of psilocyn mushrooms, one MDMA tablet and smoked a marijuana blunt laced with cocaine. In this case, psilocyn results were approximately 6X higher than the levels reported by Sticht et.al.(1). Furthermore, there are reports of smoking marijuana blunts that have elevated THC percentages. Aggressive and belligerent behavior exhibited in the above report is consistent with synergistic effects of multiple drug consumptions of hallucinogens and stimulants.

1.G.Sticht, H.Kaferstein,, *Detection of psilocin in body fluids,* Forensic Science International 113 (2000) 403-407.

Keywords: psilocin, marijuana, cocaine, mdma

TITLE: AMPHETAMINE CONCENTRATIONS IN URINE AFTER THE USE OF DEXEDRINE "GO-PILLS": COMPARISON OF SINGLE AND DOUBLE DOSES OF D-AMPHETAMINE.

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The Air Force, under the close supervision of flight surgeons, approves the use of amphetamine for both active duty and reserve Air Force aviators to counter performance problems during long military missions associated with fatigue and sleep deprivation. These pilots may also be subjected to urine drug tests, which include testing for amphetamines by DoD. Studies were conducted to determine the window of detection for amphetamine after the administration of Dexedrine ("Go-Pills") at two approved doses. Volunteers (22-58 years, mean=38.7 years) were administered a single 10mg dose of d-amphetamine (N=31), or two 10mg doses four hours apart (N=31). A urine specimen was collected from each subject just prior to drug administration and collected ad libitum thereafter for several days. Urine was screened using the KIMS immunoassay. GC/MS was performed on specimens from each subject until the amphetamine concentration fell consistently below 350ng/ml in the screen test. Based on GC/MS analysis, peak urine concentrations of amphetamine ranged from 602 ng/ml to 12,191 ng/ml with a mean of 3345 ng/ml in the single-dose study, and from 1,339 ng/ml to 15,359 ng/ml with a mean of 6076 ng/ml in the two-dose study. The time at which the highest (peak) concentration was observed ranged from 2.6 to 29.6 hours with a mean of 12.3 hours (sd=6.3) in the single dose study, and from 4.0 to 26.5 hours with a mean of 13.8 hours (sd=6.7) in the two-dose study. The time to the last identified positive (>500 ng/ml) in each subject ranged from 2.6 to 46.7 hours with a mean of 31.3 hours (sd=9.7) in the single-dose study, and from 29.1 to 71.5 hours with a mean of 44.8 hours (sd=9.3) in the two-dose study. The excretion rate half-life of amphetamine in the urine was approximately 12 to 13 hours in both test groups. These studies indicate that most pilots should be below the DoD cutoff of 500 ng/ml within 48 hours after a single 10 mg dose, and within 72 hours after the 2x10 mg dose.

Key Words: Amphetamine, Urine, Go-Pills

WORKPLACE URINE OPIATE TESTING: A CASE OF SCIENTIFIC INJUSTICE IN THE U.K.

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A successful professional accepts a supervisory nursing position, provisionally, pending a negative urine drug test. She advises that she may have taken paracetamol within two weeks prior to the testing. Unexpectedly, the subject's urine, Sample A, is found to contain a 'total' morphine concentration of 403 ng/mL. The employer, based on this 'positive' test, refuses her the position, and the company's medical review officer will hear no explanation whatsoever. Five months later the certificate with the test result of Sample A and the second urine sample, Sample B, are released to the donor. Independent analyses indicate 'free' morphine of 27 ng/mL, 'total' of 687 ng/mL and the presence of thebaine in this second sample. No codeine is detected. The total morphine values exceed the U.K. morphine limit of 300 ng/mL, yet both sample results are below the U.S. and U.S. Military limits of 2,000 ng/mL and 4,000 ng/mL respectively.

As will be discussed, selected requirements within the U.K. Guidelines for Legally Determined Workplace Drug Testing were not followed. Violations include: the lack of temperature measurements, witnessing the original sealing of the samples followed by the samples being subsequently unsealed, repackaged, and resealed with the original seals, no hand washing between various samples being handled, no disclosure of original results, no timed release of Sample B, nor the availability of declaring a 'dietary source' on the disclosure forms, amongst others. No interpretations were carried out with consultation between the medical review officer with the original toxicologist and the general medical practitioner of the donor, concerning the 'positive' result, as required. In fact, the donor was originally verbally told that 'codeine' was present in her urine via telephone.

The source of morphine was the consumption of 'Warburton's Seeded Batch Bread' containing approximately 0.5 grams of poppy seeds per slice. The donor had eaten this bread during the four days prior to the drug testing date and on many other previous occasions. Having provided the employer with this information, the company refuses to overturn its original decisions: that the donor was positive for morphine without reasonable excuse nor place her in the position for which she had been successful, despite having dismissed the original medical review officer. The donor remains unable to secure suitable professional employment in her field because on all subsequent employment applications she must indicate that she has been refused employment as a result of a positive urine drug test. Each time such violations occur in drug testing at the workplace programs, insurmountable injustices are placed on the victims, as this cases demonstrates.

Keywords: workplace, drug-testing, morphine

AUTOMATED APPROACH TO NON-NEGATIVE SPECIMEN LIST (NNSL) PRODUCTION AND APPLICATION FOR SAMHSA-CERTIFIED LABORATORIES.

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SAMHSA-certified laboratories are required to produce a Non-Negative Specimen List (NNSL) for the National Laboratory Certification Program (NLCP) on a semi-annual basis. This list must be submitted to the NLCP staff Research Triangle Institute (RTI) on a specifically formatted Excel spreadsheet. From the list, NLCP staff select those specimens whose documentation are to be reviewed by inspectors at the next scheduled on-site inspection. For ease of review, as mandated by the inspection guidelines, these documents must be organized in a manner that reflects the selected NNSL. To decrease the time needed to compile this information, we have designed an approach to automate this organization process. Our application of this approach uses SQL and Turbo Pascal computer languages and the spreadsheet Excel, with *Commercial Lab* as the Laboratory Information Management System (LIMS). First, SQL queries are run which produce text files from resident databases on the LIMS. Second, the text files are moved to a PC and imported into pre-formatted Excel worksheets, which are then forwarded to RTI. Third, the files are refined using a Turbo Pascal program and imported into Excel calendar worksheets. Calendar entries include: Access number, Batch number, CCF number and the Non-negative test for which the sample was targeted. The listings on the calendar (placed on boxes containing the sample documentation), in conjunction with the selected NNSL, organize the specimen documentation for easy retrieval and review by the inspectors. Inspectors to our laboratory have praised this organizational scheme. As pre-inspection guidelines become increasingly time consuming, this approach may be useful for other SAMSHA-certified laboratories.

Keywords: NNSL, SAMHSA, Non-negative

METHOD VALIDATION FOR THE ANALYSIS OF AMPHETAMINE, METHAMPHETAMINE, MDA AND MDMA IN URINE

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The purpose of this study is to provide the standard method for the assay of stimulants in urine, especially ATS (amphetamine type stimulants; amphetamine (AM), methamphetamine (MA), methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA)). We performed method validation for these drugs in urine according to EURACHEM Guide (A laboratory guide to method validation).

Analytical method for AM, MA, MDA and MDMA in urine was as follows. For the analysis of amphetamines in urine. immunoassay (fluorescence polarization immunoassay, TDxFLx) was used for screening test and confirmation test was performed with GC/MS. After the analysis by immunoassay, the positive specimens were alkalized with 6 M-NaOH and the analytes were extracted with ethyl acetate. After centrifugation of specimens, the supernatants were evaporated to dryness under the nitrogen stream at 45□ with vacuum. The residues were derivatized with pentafluoropropionic anhydride (PFPA). They were analyzed by gas chromatography/mass spectrometry (GC/MS). D_5-deuterated amphetamines were used as internal standards. To validate this method, selectivity, linearity of calibration, within- and between-run reproducibility (precision), accuracy, limit of detection and quantification were studied.

As a result, the calibration curves were ranged from 100 to 8,000 ng/ml with the correlation coefficients of greater than 0.9. Within- and between-run precisions were measured in three different concentrations (250 ng/ml, 500 ng/ml, 1,000 ng/ml). Coefficients of variance (CV %) of their precisions were under 10 % at all concentrations. Those accuracies (% bias) were also under 10 %. The limit of detection (LOD) and limit of quantification (LOQ) for all analytes studied were 20 ng and 100 ng, respectively. The selectivity of amphetamines in urine was observed by spiking high concentrations of those, which have similar chemical structures such as ephedrine, pseudoephedrine and norephedrine into low QC urine samples (500 ng). Those chemicals did not show any inferences in the analysis of ATS in urine by this method.

It was concluded that validation data by Eurachem guide was proved to be adequate for the analysis of amphetamine, methamphetamine, MDA and MDMA in urine samples.

Keywords; method validation, ATS (amphetamine type stimulants), urinalysis

URINARY EXCRETION OF MORPHINE AND CODEINE FOLLOWING THE ADMINISTRATION OF SINGLE- AND MULTIPLE-DOSE OF BROWN MIXTURE

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Parallel to the "poppy-seed defense" strategy commonly reported in the United States, donors of urine samples tested positive for opiates in Taiwan often claimed the consumption of Brown Mixture (BM) as the source of the observed morphine and codeine. Since BM contains opium powder (10-10.5% morphine), opium tincture (0.9-1.1% morphine), or camphorated opium tincture (0.045-0.055% morphine) and is a popular remedy, while heroin use is considered a serious criminal act, the claim of BM use has to be adequately addressed.

In this study, BM from 7 different manufacturers (5 tablets and 2 solutions) and urine samples from patient and alleged heroin users were analyzed for their morphine and codeine contents. The analytical procedure included hydrolysis, trimethylsilylation, and monitoring of the following ions designated for TMS-derivatized codeine, morphine, and nalorphine (internal standard): *m/z* 371, 356, 343; 429, 414, 401; 455, 440, 414, respectively. The first ion listed for each compound was used for quantitation using a six-point calibration protocol (50-2000 ng/mL).

The contents of morphine and codeine and their ratios ([M]/[C]) in: (a) BM tablets (n = 5), and BM solution (n = 2) available in Taiwan and (b) urine specimens collected from alleged heroin users and patients (n = 7) ingesting 1–6 tablets (or 5-20 mL solution) -- one-time single dose or three times per day for two days -- are summarized in Table 1. The contents of morphine and codeine in the tablets are very consistent, but vary considerably in the 2 BM solution. Morphine concentrations found in urine specimens collected from patients ingesting BM tablets or solution, are always <4000 ng/mL. The following [M]/[C] ratios were observed for urine specimens with morphine concentration
≥300 ng/mL: (a) <3.0 for patients ingesting BM solution; (b) >3.0 (mostly >5.0) for patients ingesting BM tablets and alleged heroin users. It appears that (a) BM ingestion (tablet or solution) is unlikely to result in morphine concentration >4000 ng/mL; and (b) [M]/[C] ratio may not be an effective parameter for the differentiation of BM tablet ingestion and heroin use.

Table 1. Morphine and codeine contents in Brown Mixture tablet, Brown Mixture solution, and urine specimens collected from alleged heroin users and patients ingesting Brown Mixture

	Brown Mixture		Urine specimen[a] collected users of		
[M]/[C]	Tablet	Solution	Tablet	Solution	Heroin[b]
N	25	10	56	68	90
Range	8.46-9.18	2.56-2.93	4.88-14.8	1.31-9.09	3.15-40.8
Mean	8.77	2.74	7.76	2.62	8.52
Std dev.	0.19	0.15	1.92	0.87	4.03

[a] Only specimens with morphine concentration higher than 300 ng/mL are included.
[b] Alleged heroin users.

Key words: Brown mixture, morphine, codeine

A MODIFIED METHOD FOR THE LIQUID-LIQUID EXTRACTION AND GC/MS ANALYSIS OF METHADONE FROM HUMAN URINE IN A CAP-FUDT CERTIFIED DRUG TESTING LABORATORY

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In order to produce forensically accurate and reproducible results in a competitive, cost-effective, and time conserving manner, we took an existing method (1,2) for the extraction of methadone from urine and made several significant modifications. The internal standard was changed from phenyltoloxamine to methadone-d9. The extraction method was changed from a solid phase/back extraction to a liquid/liquid extraction.

Four organic solvents were tested:
Solvent A comprised of (35:15:40:10) toluene:ethyl acetate:hexane:methanol.
Solvent B comprised of (25:25:50) toluene:ethyl acetate:hexane.
Solvent C comprised of (10:90) toluene:hexane.
Solvent D comprised of (5:95) isoamyl alcohol:hexane.

All solvents were evaluated in a liquid-liquid extraction procedure for quantitation ion areas 72 m/z and 78 m/z with calibrators spiked at 80 ng/mL, 200 ng/mL, and 2000 ng/mL. Area recovery studies showed that solvent B was the most desirable of the four extraction solvents. Solvent A was eliminated because it had the second lowest correlation coefficient (r^2 =0.999985), and because it contained methanol, which could cause emulsions to form. Solvent C was eliminated because it had the lowest recovery of internal standard and the largest variation in internal standard recovery. Finally, solvent D was eliminated because it had the lowest correlation coefficient (r^2 =0.999929) and the second largest variation in internal standard recovery.

Linearity studies with our previous extraction method produced an LOD (Limit of Detection) of 50ng/mL, LOQ (Limit of Quantitation) of 100ng/mL and an ULOL (Upper Limit of Linearity) of 2,000ng/mL. The new extraction method allowed this laboratory to expand our ULOL from 2,000ng/mL to 10,000ng/mL allowing for an accurate quantitation of high concentrated samples on a first time run without prior dilution. This method modification reduced the rescheduled rate from 27% to 9%.

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Keywords: Methadone, Liquid/Liquid, GC/MS

A RAPID LC/MS METHOD FOR THE DETERMINATION OF METHAMPHETAMINE/DIMETHYLAMPHETAMINE AND THEIR METABOLITES IN URINE - A STUDY OF THE CURRENT SITUATION IN HONG KONG

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Methamphetamine (MA) abuse, both in high purity "Ice" form or in tablets, continues to be one the popular drugs of abuse found in Hong Kong. Recently, some of drug seizures in crystalline or tablets received by our laboratory were found to contain N,N-dimethylamphetamine (DMA), a MA analog. The crystalline samples with DMA found were either in its pure form or mixed with MA. For tablets containing DMA, they were physically similar to those Ecstasy tablets, and the compositions were complex and usually mixed with MA, MDMA, ketamine and/or other drugs. As it is difficult to distinguish physically between DMA and MA, DMA can be mistakenly sold as MA.

It has been reported that DMA, N,N-dimethylamphetamine N-oxide (DMANO), MA, and amphetamine (A) were excreted in human urine after administration of DMA. As DMANO, one of the main and specific metabolite of DMA, is thermally labile; the use of LC/MS would allow the simultaneous detection of all four analytes without derivatization possible. In order to have a clear picture of the current state of MA/DMA abuse in Hong Kong, a rapid and sensitive LC/MS method has been successfully developed for the urinalysis of suspected drug abusers under the drug use surveillance program. LC/MS analysis was performed on a Finnigan Surveyor LC connected to a Finnigan LCQ Advantage ion trap mass spectrometer. Using solid phase extraction of urine with Alltech C18 columns followed by LC/MS analysis fitted with an Alltech Rocket Platinum EPS C-18 column and using a mobile phase (0.01M ammonium formate (pH=3): acetonitrile =77:23) at a flow rate of 0.2 ml/min, the simultaneous identification and quantitation of MA, DMA and their metabolites could be achieved within 5 min. Based on the urinalysis results, an overview of current MA/DMA abuse will be described.

Keywords: N,N-dimethylamphetamine, LC/MS, urinalysis

CONFIRMATION RATES OF INITIAL DRUG ASSAYS IN A GROUP OF HHS-CERTIFIED LABORATORIES, JANUARY 01 THROUGH DECEMBER 31, 2003
I: FEDERALLY REGULATED SPECIMENS

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As the U.S. Department of Health and Human Services (HHS) moved to expand the analytical methods, the approaches to drug detection, and the biological matrices allowed as specimens in the workplace drug testing program for Federal employees, an in-depth analysis of current practices was initiated. Of particular interest was the specificity and cross-reactivity of the immunoassays currently found in HHS-certified laboratories. The specificity of the immunoassays associated with urine drug testing has long been a subject of discussion among forensic toxicologists. While it has been known that some drug class immunoassays have very high rates for the confirmation of presumptive positives, it is also recognized that other drug class immunoassays produce a significant number of presumptive positives that fail to confirm when subjected to confirmatory testing by GC/MS. These observations led to an examination of the immunoassays currently in use with the goal of documenting the possible differences in specificities and cross-reactivities of the technologies.

The study included data from 11 HHS-certified laboratories encompassing nearly 4 million specimens tested under Federal mandate during 2003. These specimens represented between 55 to 60% of all federally regulated specimens tested in accordance with the Mandatory Guidelines for Federal Workplace Drug Testing Programs (59 Fed. Reg. 29908-29931, June 9, 1994 and 63 Fed. Reg. 63483-63484, November 13, 1998) during 2003. The data were obtained from laboratories that used CEDIA, EIA and KIMS technologies as a primary initial test. Some laboratories conducted additional screening of presumptive positives with FPIA as a second initial test. Summaries of specimen testing and confirmation rates are presented in the tables below. The confirmation rates are expressed as percent of the presumptive positives confirmed by GC/MS for each drug class. The mean, lowest and highest laboratory confirmation rate for each drug class are also provided.

	Amphetamines	BZE	Opiates	PCP	THC-COOH
Specimens Tested	3,939,614	3,946,445	3,937,611	3,937,611	3,946,445
Presumptive Positives	21,577	23,570	21,586	1,772	54,578
Confirmed Positives	11,715	22,920	6,550	1,229	48,458

Initial Test Assay Confirmation Rates	Amphetamines ($1^{st}/2^{nd}$ Test)	BZE	Opiates	PCP	THC-COOH
Mean Rate	51.9%/82.8%	98.1%	30.2%	69.7%	91.0%
Lowest Rate	37.4%/81.3%	91.1%	17.3%	51.6%	73.0%
Highest Rate	77.8%/84.3%	99.9%	55.9%	91.0%	98.8%

This study evaluated the presumptive positive rates and the confirmation rates for primary initial tests by immunoassay method as well as paired immunoassay methods (primary initial test plus second initial test). The results were examined with consideration of assay cross-reactivity and specificity. As expected, some assays and technologies appear to better identify specimens containing analytes of interest at or above the administrative cutoffs required by the Mandatory Guidelines for Federal Workplace Drug Testing Programs. While the study assesses current capabilities of existing technologies from a large population of "real" federally regulated workplace specimens, it also provides information that may be useful in formulating future guidelines by which newer technologies and approaches may be evaluated.

Keywords: HHS-certified laboratories, Immunoassay confirmation rates, calendar year 2003

CONFIRMATION RATES OF INITIAL DRUG ASSAYS IN A GROUP OF HHS-CERTIFIED LABORATORIES, JANUARY 01 THROUGH DECEMBER 31, 2003
II: NON-REGULATED SPECIMENS

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The specificity of immunoassays associated with urine drug testing has long been a subject of discussion among forensic toxicologists. While it has been known that some drug class immunoassays have very high rates for the confirmation of presumptive positives, it is also recognized that other drug class immunoassays produce a significant number of presumptive positives that fail to confirm when subjected to confirmatory testing by GC/MS. These observations are further confounded as initial and confirmatory drug test cutoff concentrations change and as the number of drug analytes in confirmatory panels are broadened. These observations led to an examination of the immunoassays currently in use at drug testing laboratories. The goal of this study was to document the possible differences in specificities and cross-reactivities of the technologies with multiple cutoffs in both the initial and confirmation testing procedures in addition to possible variability in the analytes defined in the confirmatory panels.

The study included data from 10 laboratories certified by the U. S. Department of Health and Human Services (HHS) encompassing over 10 million specimens tested during 2003. These specimens were not subject to the criteria of the Mandatory Guidelines for Federal Workplace Drug Testing Programs. The data was obtained from laboratories that used CEDIA, EIA and KIMS technologies as a primary initial test, with varying initial and confirmatory test cutoffs. Some laboratories conducted additional screening of presumptive positives with a second initial test. Summaries of specimen testing and confirmation rates are presented in the tables below. The confirmation rates are expressed as percent of the presumptive positives confirmed by GC/MS for each drug class. The lowest and highest confirmation rate for each "initial/confirmatory testing cutoff pair" identified is provided.

	Amphetamines	BZE	Opiates	PCP	THC-COOH
Specimens Tested	10,142,363	10,136,424	10,117,626	10,006,492	10,188,976
Presumptive Positives	101,653	97,891	103,081	5,838	347,905
Confirmed Positives	57,283	96,283	36,821	3,389	326,039

	Barbs	Benzos	Methadone	MQL	Propoxyphene
Specimens Tested	4,585,890	4,271,398	3,943,430	2,504,097	3,852,794
Presumptive Positives	15,281	47,639	10,305	56	28,188
Confirmed Positives	13,729	28,260	9,248	4	26,045

Initial Test Assay Confirmation Rates	Amphetamines	BZE	Opiates	PCP	THC-COOH
Lowest Rate	3.4%	80.5%	25.0%	59.0%	60.8%
Highest Rate	97.2%	99.3%	71.8%	96.4%	100.0%

Initial Test Assay Confirmation Rates	Barbs	Benzos	Methadone	MQL	Propoxyphene
Lowest Rate	76.1%	45.9%	69.8%	NA	22.7%
Highest Rate	100.0%	83.7%	100.0%	8.3%	100.0%

This study evaluated the presumptive positive rates and the confirmation rates for primary initial tests by immunoassay method. The results were examined with consideration of assay cross-reactivity and specificity. As expected, some assays and technologies appear to better identify specimens containing analytes of interest above the defined cutoffs. While the study assesses current capabilities of existing technologies from a large population of "real" specimens, it also provides information that may be useful in formulating future guidelines by which newer technologies, additional drug classes and additional analytes may be evaluated.

Keywords: non-Regulated testing, HHS-certified laboratories, Immunoassay confirmation rates, Calendar Year 2003

DETERMINATION OF BENZODIAZEPINES IN HUMAN URINE USING SOLID-PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ELECTROSPRAY IONISATION TANDEM MASS SPECTROMETRY

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Aims: A liquid chromatography-tandem mass spectrometry (LC/MS/MS) method has been developed and validated for the determination of benzodiazepines registered in Norway and/or their metabolites in human urine. These compounds are frequently associated with misuse. The following compounds are included: 7-aminonitrazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, alprazolam, alphahydroxyalprazolam, oxazepam, 3-OH-diazepam and *N*-desmethyldiazepam. The method was evaluated by analysing urine specimens from prison inmates, suspected of drug abuse.

Methods: Urine samples (0.5 ml) were hydrolysed with ß-glucuronidase (from *Patella vulgata*) at 60 °C for 2 hours before solid-phase extraction with a polymer-based mixed-mode column (Oasis MCX). Chromatographic separation of extracts was achieved using a Waters Symmetry C18 (2.1X100 mm, 3.5 µm) column with a flow rate at 0.3 ml/min with gradient elution. The analyses were performed on a Waters Alliance 2695 system in combination with a Waters Quattro Ultima Pt tandem-quadropole mass spectrometer equipped with a Z-spray electrospray interface. Positive ionization was performed in the MRM (multiple reaction monitoring) mode. Two transitions were monitored for the analytes, and one for the internal standards. Deuterated analogues were used as internal standard for all analytes except for 7-aminonitrazepam and alphahydroxyalprazolam, which were quantified using 7-aminoclonazepam-d4 and alprazolam-d5, respectively.

Results: The concentration range was 0.1-8.0 µM for 7-aminonitrazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, alprazolam, alphahydroxyalprazolam and 0.5-40 µM for the other compounds. The average recovery of the analytes ranged from 56-83 %. The between-day relative standard derivation of the method ranged from 3 –12 %. The limits of quantification were found to be between 0.002 and 0.01 µM.

Conclusion: The LC/MS/MS method proved to be robust and specific for the determination of benzodiazepines in urine. The method developed offers significant efficiency advantages in our routine laboratory replacing two chromatographic methods, which involved time-consuming derivatisation techniques. (HPLC-fluorescence, GC-MS). In addition, inclusion of new benzodiazepines is more convenient due to high specificity and flexibility of the new method.

Keywords: Benzodiazepines, urine, LC/MS/MS

USE OF COMPOUNDS ALTERING VIGILANCE PERFORMANCE: PRELIMINARY RESULTS OF PREVALENCE IN HAULAGE DRIVERS IN THE NORD-PAS-DE-CALAIS REGION (FRANCE)

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Introduction: In 1995, a French study showed that the number of users of compounds that could alter vigilance performance was significantly more important in a population of workers in security posts than in the general working population (1). Moreover, even if the number of road accidents involving haulage drivers has decreased since 1985, their severity is greater. A multicentric study was initiated by a local group « Toxicomanie et Travail » on haulage drivers from different cities in the Nord-Pas-de-Calais region (France). The objectives of this study are the standardisation of occupational medicine practices in order to define a harmonized policy for prevention and screening, to validate analytical screening methods, as well as the course of action in the case of positive detection.

Methods: 1000 haulage drivers were included in the study. Occupation was the only criterion of selection. Urine samples were collected anonymously. Information collected included: haulage area, age, gender, nature of appointment with occupational practitioners and day of the sampling. A urine screen (test Triage^{TM}8, BMD) was performed for methadone (cut off = 300 ng/mL), benzodiazepines (300 ng/mL), cocaine (300 ng/mL), amphetamine derivatives (1000 ng/mL), opioides (300 ng/mL) and cannabis (50 ng/mL). Buprenorphine detection was achieved with Elisa method (Microgenics). All positive results were confirmed by GC-MS or HPLC-MS. Alcohol levels were determined by an enzymatic method (Dade Behring).

Results: Up to February 2004, 707 samples were collected. 99% are men and participants were on average 36.4 +/- 9.9 years old. Results expressed as percentage of positives were : opioides (4.81%), cannabis (9.05%), cocaine (0.14%), amphetamine derivatives (0%), buprenorphine (1.98%), methadone (0.56%), benzodiazepines (0.42%), alcohol (5.51%). The 34 positive cases for opioides were distributed as follows: 6-MAM 0 ; morphine 10 ; codeine 11 ; pholcodine 20. THC-COOH was the only compound identified in urine samples from individuals tested positive for cannabis.

Conclusion: Only legal opioid drugs were identified. They correspond to antitussive medications that are broadly prescribed during the winter period (time of the urine collection). The lack of sensitivity and specificity of the immunologic test could explain the low percentage observed for benzodiazepine positive cases. Our results are in good agreement with the results of the previously mentioned study of Haguenoer et al., (1995) in the North of France, for the use of cannabis and alcohol by workers in security posts. In contrast, the number of positive cases for amphetamine derivatives, opioides and benzodiazepines was less. Other molecules were not investigated in the previous study of Haguenoer et al.. Considering these preliminary results, it would be of great interest to extend the screening to other psychoactive molecules by using a more efficient screening method such as HPLC-DAD.

References: 1. Haguenoer J.M. et coll. Prévalence des conduites toxicophiles en milieu professionnel : une étude dans la région du Nord Pas de Calais. Bulletin du Conseil départemental du Nord, Ordre des Médecins, 1997 ; 80 : 11-15.

Keywords: vigilance, haulage drivers, illicit substances

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Keywords: vigilance, haulage drivers, illicit substances

SCREENING OF BUPRENORPHINE IN URINE OF SUSPECTED ABUSERS BY ELISA

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Introduction: Buprenorphine is a synthetic thebaine derivative that has both analgesic and opioid antagonist properties. As an analgesic, it is 25 to 40 times more potent than morphine, and has a slower onset of pain relief and longer duration of action. Hence it is used for the treatment of chronic pain, and in treatment of heroin addiction as an alternative to methadone. In human, buprenorphine is metabolised primarily by N-dealkylation to norbuprenorphine. Both the metabolite and the parent drug undergo extensive conjugation to glucuronides that are excreted in urine. It is known that buprenorphine has been abused by heroin addict; therefore there is a need for the laboratory to develop a screening method for the suspected abusers.

Aim: The purpose of this study is to evaluate the use of an Enzyme Linked Immunosorbent Assay (ELISA) test kit in the screening of buprenorphine in urine to detect suspected abusers.

Method: The ELISA test kits were purchased from Neogen Corp. USA. The assay principle is based on the competitive binding between the free drugs in the urine and the drug enzyme conjugates for antibodies, which are coated onto the 96-well microplate. After incubating the urine and drug enzyme conjugate in the wells for 45 min at room temperature, the wells were washed with buffer, and K-blue substrate was added to each well. After a further 30 min of incubation, Red Stop solution was added to each well to stop the reaction. Multiskan Ascent Microplate Photometer was used to measure the absorbance at wavelength of 650 nm. The color intensity of each well is inversely proportional to the concentration of the drug in the urine. Confirmatory test, using Gas chromatography-mass spectrometry (GC-MS) was also carried out on all the urine specimens to compare with the screening results.

Results: The linearity of the ELISA kit was found to be up to about 5 ng/ml of buprenorphine. The coefficients of variation (CVs) of the within-day and between-day variations for buprenorphine ranged from 2.0% to 6.7% and 8.3% to 19.1% respectively. Norbuprenorphine was found to cross react to certain extend. No cross-reactivity was found with other common drugs of abuse such as morphine, codeine, hydrocodone, hydromorphone, 11-nor-Δ^9-THC-9-carboxylic acid, cocaine, benzoylecgonine, amphetamine, methampethamine, N,α-Dimethyl-3,4-(methylenedioxy)phenethylamine (MDMA), α-Methyl-3,4-(methylenedioxy)phenethylamine (MDA), N-Ethyl-α,methyl-3,4-(methylenedioxy)phenethylamine (MDEA), ketamine and norketamine. Out of the 37 urine specimens that were collected from suspected abusers, 17 were found to be screened positive, using the cut-off at 1 ng/ml. The linearity range of buprenorphine was found to be up to 50 ng/ml. The LOQ was found to be 0.25 ng/ml. The results from the confirmatory test are also discussed.

Conclusions: The use of ELISA test kit was found to be a simple, rapid, specific and effective method for the screening of buprenorphine in urine. It helps to minimize the cost of the GC-MS test in a mass screening exercise.

Keywords: ELISA, Screening, Buprenorphine

A COMPARISON OF THE MICROGENICS DRI® OPIATE AND MICROGENICS DRI® OXYCODONE IMMUNOASSAYS WITH GAS CHROMATOGRAPHY/MASS SPECTROMETRY FOR THE DETECTION OF OPIATES AND OXYCODONE IN URINE

Joseph R. Monforte, Ph.D., DABFT *, Ronald Backer, Ph.D., DABFT and Alphonse Poklis, Ph.D., DABFT

Oxycodone is a semi-synthetic opioid prescribed for pain management of moderate to severe pain. It is considered to have a high abuse potential and is prescribed as oxycodone HCl in preparations such as Oxycontin® (a controlled- release preparation), Percodan® (with aspirin) and Percocet® (with acetaminophen). Oxymorphone and noroxycodone are metabolites of oxycodone. Oxymorphone is a potent analgesic while noroxycodone is relatively inactive.

Various immunoassays are available for the detection of opiates in urine. These assays generally have lower sensitivities to oxycodone and oxymorphone. Consequently, oxycodone may not be detected employing available opiate assays, even when taken under abused conditions. The Microgenics DRI® Oxycodone Assay uses specific antibodies that can detect oxycodone and oxymorphone without significant cross-reactivity from other opiates. The assay employs either a 100ng/mL or a 300ng/mL cutoff.

Seventy specimens received from pain management patients were tested for opiates with the Microgenics DRI® Opiate and Microgenics DRI® Oxycodone Immunoassays, and gas chromatography/mass spectrometry. Immunoassay reagents were employed as directed by the manufacturers. The cutoff for the Microgenics DRI® Opiate Assay was 300 ng/mL, and the cutoff for the Microgenics DRI® Oxycodone Assay was
100 ng/mL. The GC/MS SIM procedure employed hydrolysis of the specimen, treating the urine with hydroxylamine, a solid phase column extraction, and formation of the BSTFA derivative. The method detects oxycodone, oxymorphone, hydrocodone, morphine and codeine. The limit of detection and limit of quantitation is 20 ng/mL for each opiate.

Of the seventy specimens tested by the Microgenics DRI® Opiate Assay, fifty-three (53) specimens tested positive and seventeen specimens tested negative. All seventeen negative specimens were confirmed negative for the opiates included in the GC/MS SIM procedure and all seventeen specimens tested negative for oxycodone using the Microgenics DRI® Oxycodone Assay. An opiate was detected by GC/MS in all of the specimens testing positive by the Microgenics DRI® Opiate Assay with four exceptions.

Of the seventy specimens tested by the Microgenics DRI® Oxycodone Assay, twenty-four specimens tested positive for oxycodone. All twenty-four specimens tested positive for oxycodone by GC/MS, and twenty-two of the specimens also contained oxymorphone. One specimen, which did not contain a reportable oxymorphone concentration, also contained morphine (4,679 ng/mL). One specimen tested one point below the Microgenics DRI® Oxycodone cutoff and contained morphine (18, 522 ng/mL) but no reportable oxycodone. All forty-six specimens, which tested negative for oxycodone with the Microgenics DRI® Oxycodone Assay, also tested negative for oxycodone and oxymorphone by GC/MS.

The results obtained with this limited number of specimens indicates that the Microgenics DRI® Oxycodone Assay is highly specific for oxycodone and, consequently, has the potential to be very effective in discriminating oxycodone from other opiates which may be present in a urine specimen.

Keywords: Oxycodone, Immunoassay, Gas Chromatography/Mass Spectrometry

IDENTIFICATION OF CHLORINATED 3,4-METHYLENEDIOXYMETHAMPHETAMINE IN ILLICIT DRUG ABUSER URINE

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Aim: The aim of this study was to identify 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and chlorinated 3,4-methylenedioxymethamphetamine (Cl-MDMA) using combined thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS). The compound was identified jointly with MDMA in illicit drug abuser urine. The urine specimen has yielded positive EMIT immunoassay screenings for cannabinoides, amphetamines and cocaine. GC-MS confirmation of amphetamines has revealed the presence of amphetamine, methamphetamine, ephedrine, norephedrine, 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine (MDA).

Methods: Thin layer chromatography: In the basic extract a routine analysis of urine by TLC for amphetamines using fractional diethylether liquid/liquid extraction identified ephedrine, the major component MDMA and an unknown compound. In the TLC system our unknown compound behaved differently than the MDMA. The TLC plate with silica stationary phase was used (Merck), eluent ethylacetate ethanol ammonia (36:2:2). *Gas chromatography-mass spectrometry:* The unknown compound isolated by TLC technique was analysed by positive chemical ionization (PCI). The base peak *m/z* 228 (MH^+) exhibited a characteristic A+2 isotopic cluster indicating the presence of a monochloro-substituted compound. The molecular ion of Cl-MDMA was found at 227 *m/z*. The MS spectrum of the unknown compound in electron ionization (EI) exhibited characteristic A+2 chlorine isotopic cluster typical for ion at *m/z* 169 (*m/z* 135-1+35) and for molecular ion at *m/z* 227 (*m/z* 193-1+35). To confirm Cl-MDMA structure, the unknown compound isolated by TLC technique was acetylated, trifluoroacetylated, silylated, heptafluorobutyrylated and analyzed by GC-MS. The EI-MS spectra of MDMA and Cl-MDMA after derivatization exhibited structural similarities. The EI-MS spectrum of MDMA contained ions 193, 177, 135, 77, 58 [1]. The EI-MS spectra of derivatized Cl-MDMA exhibited characteristic A +2 isotopic cluster for ion at *m/z* 169 (*m/z* 135 –1 +35) for all used derivatization methods mentioned above. Another isotopic A + 2 cluster was created in all molecular ions of each derivatized chlorinated MDMA: acetylated Cl-MDMA had molecular ion at *m/z* 269 (*m/z* 227 – 1 + 43), silylated Cl-MDMA had molecular ion at *m/z* 299 (*m/z* 227 – 1 +73), trifluoroacetylated Cl-MDMA had molecular ion at *m/z* 323 (*m/z* 227 – 1 + 97), heptafluorobutyrylated Cl-MDMA had molecular ion at *m/z* 423 (*m/z* 227 – 1 +197). Next joint cluster at *m/z* 196 (162 – 1 + 35) occurred in acetylated, trifluoroacetylated and heptafluorobutyrylated derivates with the exception of silylated derivates. GC-MS analysis were performed using Hewlett Packard 5890 Gas Chromatograph with 5890 autosampler coupled to a Hewlett Packard 5972 MS detector and Finnigan MAT Magnum. CI-GC-MS Finnigan MAT Magnum conditions were as follows: ionization gas methanol vapour, ionization energy of electrons 70 eV, specific conditions for ion trap CI/MCI parameters: CI maximum ionization time 1500 µsec, CI maximum reaction time 100 msec, CI ionizatin storage level 82 dacs, CI reaction storage level 164 dacs, reagent ion ejection level 85 amu, MCI ionization time 100 µsec, reagent ion ejection adjust 100 %, reagent reaction time 5400 µsec.

Conclusion: A chlorinated MDMA was identified after derivatization of unknown compound isolated by TLC technique. With using literature source [2] and interpretation our mass spectra of heptafluorobutyrylated Cl-MDMA we can declare that unknown compound should be 6-Cl-MDMA. 6-Cl-MDMA is listed in New synthetic drugs reported in Europe and the USA since the mid-1990s in joint action of The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)[3].

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Keywords: chlorinated MDMA, GC-MS analysis

TESTING MEDICAL PROFESSIONALS FOR AN EXPANDED MENU OF DRUGS- A PRELIMINARY SUMMARY OF POSITIVE FINDINGS

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Testing medical professional for drugs of abuse presents a challenge because of their access to drugs of choice that are not included in the scope of most drug testing menus. To assist substance abuse recovery programs, designed to allow medical professionals to continue to practice in their field of expertise, we developed an expanded menu of analytes that includes many of the known abuse drugs encountered in the medical professional population. The positive results data was summarized in three drug groups: Group 1 includes those analytes contained in a SAMHSA regulated test menu; Group 2 includes additional opiates and amphetamine like compounds not covered in the SAMHSA scope, benzodiazepines, propoxyphene, barbiturates, and methadone; Group 3 includes (cut-off concentration in ng/mL) nor-alfentanil (0.5), butorphanol (20), nor-fentanyl (0.5), ketamine (100), MDMA (200), nor-meperidine (200), nalbuphine (8), nor-sufentanil (0.5) and tramadol (500). Group 1 and 2 analytes were screened by EMIT while group 3 analytes are screened by LC/MS/MS. All screens were confirmed by either GC/MS or an alternate LC/MS/MS procedure. The confirmation menus were extensive with low concentration cut-offs. Ethanol and ethyl glucuronide were not included in this study.

A total of 5,817 samples from medical professionals were tested for group 1 and 2 drugs. The number of positive drugs findings for the SAMHSA drugs include in group 1 was 100, with amphetamine at 56 and opiates at 34 representing most of the positive findings. With the group 2 drugs there were an additional 386 positive drug findings with 193 benzodiazepines findings and the expanded opiates panel (excluding morphine and codeine) accounting for 134 of the positive findings. A total of 4,043 samples were tested for group 3 analytes, and there were 186 positive drug findings. Tramadol, meperidine, and nalbuphine were the most frequently reported positive findings in the group 3 drugs, accounted for 120, 30 and 14, respectively.

Drug Groupings	Positive Drug Findings/ Drug Group	Cumulative Positive Drug Findings
(Group 1) SAMHSA Drugs	100 of 5817 samples	100
(Group 2) expanded EMIT panels	386 of 5817 samples	486
(Group 3) LCMSMS panel	207 of 4043 samples	693

These preliminary finding support the use of expanded testing menus when testing medical professionals who are in drug recovery programs. Caution must be used when interpreting these findings since some positive findings were listed as prescribed medications.

Keywords: medical professional, positive-rate, LC/MS/MS.

LINEAR RELATIONSHIPS OF Δ-9-TETRAHYDROCANNABINOL METABOLITES IN URINE

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Many studies have focused on the metabolic patterns of marijuana and the relationship these compounds may have to possible time of cannabis exposure, based on their concentrations in urine samples. The objective of this study was to investigate the linear relationships of a group of Δ^9-tetrahydrocannabinol metabolites in random urine samples (n=95). In this study, gas chromatography-mass spectrometry (using SIM profiles) was used to monitor and quantify 11-nor-Δ^9-carboxy-9-tetrahydrocannabinol (THCCOOH), 11-Hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), Δ^9-tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD). Deuterated THCCOOH-d_3 and 11-OH-THC-d_3 were used as internal controls. The random urine samples were collected and submitted to the Montana State Crime Lab from adult probation & parole, Pre-release centers, and Youth Court cases. Since these are random urine samples, there was no way to definitely determine time of marijuana use. The results of this study indicate significant linear relationships between some of the metabolites of THC, and also the ability to detect CBN and CBD in random urine samples. The data presented will provide a collection of THCCOOH concentrations in urine with respective comparisons to the other metabolites studied.

Keywords: Δ-9-Tetrahydrocannabinol metabolites, Urine, GC/MS

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COMPARISON STUDY OF SPE AND HS-SPME IN THE DETERMINATION OF METHADONE AND ITS METABOLITES EDDP AND EMDP IN HUMAN HAIR USING GC-MS

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The objective of this research was to develop and optimize a robust head-space solid phase microextraction for the extraction of methadone and its metabolites from hair and to compare this method to a pre-existing solid phase extraction method. Methadone (6-dimethylamino-4,4-diphenyl-3-heptanone hydrochloride) is a synthetic opioid agonist with characteristics similar to those of morphine, including actions on the central nervous system and organs composed of smooth muscle. Although structurally it has no resemblance to morphine, its analgesic and spasmolytic properties have allowed it to be used as a substitute for heroin in opiate addiction programmes.

Aliquots of blank hair (10mg) were spiked with methadone and EDDP at two different concentrations and incubated in sodium hydroxide solution 1 *M* (1ml) with anhydrous sodium chloride. This was carried out in 4 ml amber screw-top vials with a PTFE/silicone septum. The vial was preheated for 20 min under agitation. The needle probe holding the 100μm polydimethylsiloxane (PDMS) fibre was then pushed through the septum and the fibre was immersed in the specimen for 20 min at a temperature of 75°C while agitated. The fibre was then transferred to the GC inlet. The injection port (operated in split/splitless mode) was set to 225°C and the purge time to 3 min. The column temperature was initially held at 80°C for 2 min, then increased to 280°C at 10°C/min and held for 5 min.

This SPME method was compared to the following SPE method. Aliquots of spiked hair (10mg) were incubated in methanol for 18h at 37°C. This methanol was then removed and the sample was sonicated with more methanol. The methanolic fractions were combined and evaporated under nitrogen and reconstituted in distilled water and phosphate buffer, pH 6. The samples were homogenised and applied to preconditioned Clean Screen columns. These were selectively washed with various solvent and aqueous mixtures. Methadone and EDDP were extracted using dichloromethane/isopropanol/concentrated ammonia (78:20:2). These fractions were then evaporated to dryness under nitrogen and reconstituted in 25 μl of ethyl acetate. They were then analysed using the same GC-MS chromatographic conditions. Relative recoveries of methadone and EDDP from hair were determined by comparing the peak heights obtained from spiked hair with unextracted standards and both were determined to be above 5%. The HS-SPME method proved to be fast and efficient and also has the advantages of being solvent free and producing cleaner extracts.

This paper presents a comprehensive, comparative study of both SPE and HS-SPME of methadone and EDDP from human hair and will report on the advantages and disadvantages of both methods.

Keywords: Methadone, Solid Phase Extraction, Solid Phase Microextraction

DRUG ANALYSIS IN HAIR: COMPARISON OF CRYOGENIC GRINDING AND CUTTING FOR SAMPLE PREPARATION.

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Sample preparation is potentially the most important step in an analytical procedure. The forensic toxicology laboratory at Glasgow University has established methods for the extraction of drugs from keratinous matrices by cryogenic grinding (nail) and by cutting with scissors (hair) (1,2). To date, only one report has compared drug extracts obtained from cut and ground hair. Eser et al. found that both the qualitative and quantitative results from ground hair were better than from cut hair (3). In this report, two methods of sample preparation for the analysis of drugs of abuse in hair are compared, namely cutting and cryogenic grinding of hair. The drugs analysed were morphine, 6-monoacetylmorphine, codeine, dihydrocodeine, methadone, EDDP, diazepam, cocaine and ecgonine methyl ester.

Samples of hair from 20 drug-related deaths were used for the evaluation. These were collected at autopsy by plucking from the occipital region of the scalp and the root balls were removed prior to analysis. Drug-free hair was obtained from laboratory personnel for use as a control and for preparation of standards. Each sample was washed with SDS (0.1% w/v), DI water and dichloromethane before being dried at room temperature overnight. Each sample was weighed and separated into two portions of equal size that were subsequently minced with scissors or ground, respectively. Mincing was carried out in screw-cap tubes with long-blade scissors for 1 minute. Grinding was carried out in liquid nitrogen using a Glen Creston Model 6750 freezer mill equipped with micro-inserts. The optimum grinding cycle was established as 2 x 2 min by SEM examination of the powders obtained following various grinding periods. The ground hair was recovered from the grinding tube and reweighed.

Hair standards were prepared by adding solutions containing known amounts of drugs to drug-free hair and allowing it to dry. After addition of deuterated internal standards, hair standards and the case hair samples (up to 20 mg) from both series were suspended in methanol (1 ml), sonicated (15 min) and left for 12 hours at 45°C. The methanol was removed from the samples to clean vials and evaporated under a flow of nitrogen at room temperature. The mixture was redissolved in 4 ml phosphate buffer for SPE with Bond Elut Certify LRC cartridges. Extracts were derivatised at 70 °C for 20 min using 50 µl BSTFA containing 1% TMCS and analysed by GC-MS using a Thermo-Finnigan Trace instrument fitted with a 30 m x 0.25 mm x 0.25 µm HP-1 column.

The cryogenic grinding device worked effectively and produced small hair particles (19-57 µ). However specimen losses (up to 50%) during the procedure were significant. The grinding method was time consuming and, coupled with the use of liquid nitrogen, made grinding a less cost-effective procedure. Comparison of the extracts from cut and ground hair by GC-MS indicated that grinding resulted in more matrix interferences and also that ground hair was more likely to produce a false negative result. For example in the case of methadone five samples were identified as containing methadone using cut hair but only one of these samples was identified as containing methadone using ground hair. However, ground hair gave higher recoveries of drugs than cut hair, after correction for sample losses, in many cases up to twice the amount of drug was extracted from ground hair.

The results confirmed the findings of Eser et al. that the ground hair was best in terms of quantitative analysis but the findings in terms of the qualitative analysis showed clearly that cut hair is the best preparation technique. Further work will indicate if grinding will permit the use of a shorter extraction period than cut hair.

Keywords: Hair Analysis, Cryogenic Grinding, Drugs of Abuse

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AMPHETAMINE BINDING TO SYNTHETIC MELANIN AND SCATCHARD ANALYSIS OF BINDING DATA

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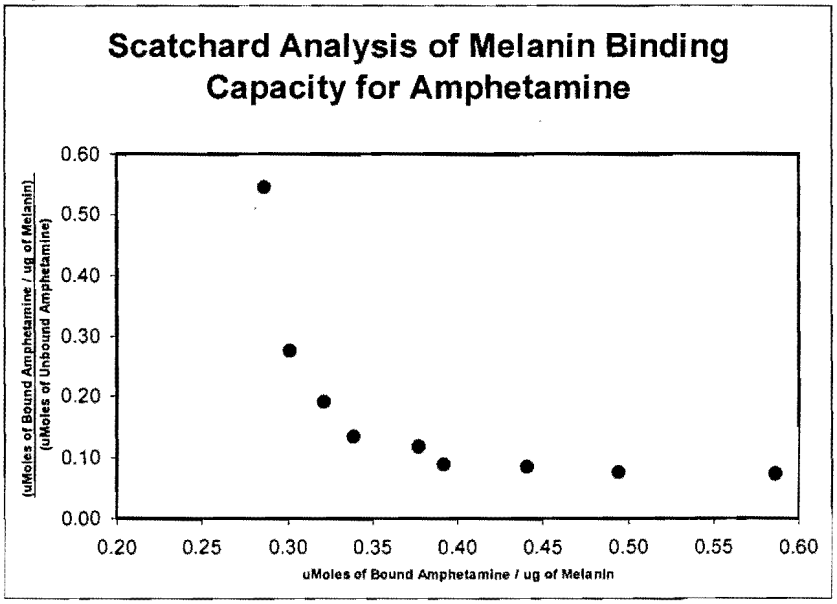
The determination of drug use and drug history has historically been carried out through the analysis of biological fluids (blood and urine). In the past decade, hair analysis has been exceedingly used in order to determine recent past drug use as well as long-term drug histories through the use of segmental analysis. In order to fully understand the implications of a positive hair test result, it is important to understand how the drugs are incorporated into hair and how they bind. Keratin, melanin and lipids are possible drug binding sites in hair. Keratin and melanin have many polar groups which serve as attachment points for the drugs. Previous research into drug-hair binding has shown that different amounts of drug bind in hair of different colours. There are no structural differences in hair of different colours other than in the type and content of melanin present. For this reason, this investigation focuses on amphetamine binding to synthetic melanin in order to help to explore the possible binding mechanisms.

The binding study was carried out at room temperature in the presence of light and oxygen using 0.1 M phosphate buffer at pH 7.4. Both compounds were found to be stable under these conditions. Amphetamine-d-sulphate and melanin, synthesized by the oxidation of tyrosine, were purchased from Sigma Chemical Company. As melanin is only sparingly soluble in buffer, it was solubilised in dimethyl sulphoxide (DMSO) prior to dilution with buffer. An indirect spectroscopic method was used by measuring the decrease in absorbance of amphetamine as the concentration of melanin was increased. Aliquots of amphetamine were titrated against the melanin solution. Samples were incubated for 45 minutes with constant shaking. Background correction was done at each step using the same amount of melanin as present in the sample for matrix matching. The interaction was monitored using a Genesys 6v, UV/Vis spectrophotometer in scanning mode from 250-264 nm focussing on changes in the amphetamine spectral fingerprint at 257.2 nm. Positive controls were prepared using the same amount of amphetamine as in the sample with the addition of buffer instead of melanin at each step. Buffer was used for the background correction to run positive controls.

To determine the classes of binding sites, association/dissociation constants and the binding capacity of melanin, the data were analysed by Scatchards' method (Scatchard *et al.*, 1949). As the molecular weight and the structure of melanin is still not clear, the number of binding sites can not be calculated directly as an integer. Instead, the ratio of the number of μmoles of drug bound and the dry weight of melanin in μg is considered. This study has proved that amphetamine binds to synthetic melanin *in-vitro*. Data analysis from the Scatchard method has yielded a curvilinear plot with upward concavity. The curvilinear plot indicates multiple binding sites and the upward concavity indicates negative co-operativity.



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Keywords: Hair, Melanin, Scatchard Analysis

ANALYSIS OF TRACE METAL CONTENT IN HUMAN HAIR USING LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY (LA-ICP-MS)

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Measuring the trace element content in human hair is traditionally done by acid digestion and analysis by atomic absorption or Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Acid digestion of samples can introduce contamination, which then lowers the accuracy of the analytical method. In addition to the contamination issues, acid digestion under trace metal clean conditions can be labor intensive and costly. Laser ablation (LA) is a sample introduction technique that, when combined with the ICP-MS, has the potential to provide accurate metal concentration data.

Here we present method development data comparing traditional acid digestion liquid-based ICP-MS to LA-ICP-MS on two certified hair standards (International Atomic Energy Agency Reference Material Human Hair IAEA-085 and The People's Republic of China Reference Material Human Hair GBW 07601) and laboratory internal standard (USGS basalt standard BCR-2). The two sample preparation techniques that were compared were acid-digestion and solid sampling via laser ablation. Internal standards were used to calibrate both liquid and solid analysis techniques to monitor instrument drift (liquid – ^{115}In; solid-total Si in ppm). The concentrations of Fe, Zn, Ca, Cu, Mg and Mn of each sample were compared, using the known concentrations from each hair standard.

Following the analysis of the BCR-2 standards and samples, we found that LA-ICP-MS analysis is reproducible only within an error of 20%. This could be due to matrix interferences (organic v.s. silicate) which were not corrected for in this preliminary study. Overall laser ablation is an excellent tool for trace metal analysis providing accurate element ratio data (eg; Cu/Ca) but not concentrations at this time. Future studies will focus on the development of a matrix matched solid standard mitigating matrix interferences.

Keywords : Trace Analysis, LA-ICP-MS, Hair

METHOD VALIDATION AND DETERMINATION OF MDMA AND MDA IN HAIR

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Recently, MDMA and MDA have been used as a recreational drug among young people in Korea. MDMA is a substitute for methamphetamine (MA), and has recently received much attention from media and law enforcement as a novel and popular psychoactive agent. As a result of the recent interest in designer drug, the abuse of MDMA and MDA has increased and there were cases in which both MA and MDMA were detected in hair samples. Therefore it was necessary for us to establish the detection method of MDMA and MDA in hair samples. The method validation and uncertainty measurement were also studied and described. The prevalence of MDMA was surveyed in hair specimens submitted for drug testing by this method.

MDMA, MDA, MDMA-d_5, and MDA-d_5 were purchased from Radian International LLC (Austin, TX, USA). Hair samples were collected from 791 subjects who were suspected MDMA and MDA users. After washing, the hair was cut into small pieces of less than 1 mm and incubated. SPME extraction was applied and extracts were derivatized by trifluoroacetic anhydride (TFA). Gas chromatograph-mass spectrometry (GC/MS) analysis was performed under selective ion monitoring (SIM) conditions for the identification and quantitation of MDMA and MDA.

Limit of detection (LOD) and limit of quantitation (LOQ) of MDA and MDMA were 0.125 ng/mg, 0.25ng/mg respectively. The linear calibration curve ranged from 0.25 to 10 ng/mg with the coefficient determination of R2 >0.99 . Within- and between run precisions were measured in three concentrations (low 0.8 ng/mg, med 4 ng/mg, high 8 ng/mg). The specificity in blank hair and dyed artificial hair was studied. The high concentration of ephedrine, norephedrine, pseudoephedrine, nicotine, caffeine, methoxyphenamine, phenterminein in low QC hair samples (0.8ng/mg) didn't show any interference in this method. To value the confidence of the method, uncertainty was performed at the cut-off level 5 ng/mg hair samples. The measurement in uncertainty of MDMA was ±0.22 ~0.30 (95% confidence interval, k=2). Among 791 hair samples studied, 44 hair samples were positive for MDMA or MDA (5.6 %). The concentration of MDA was low in the hairs where both MDMA and MDA were detected indicating MDA was the metabolite of MDMA, while in 4 specimens the level of MDA was very high with no MDMA indicating MDA was administered. However in urine samples from same 44 subjects showed 35 were negative for MDMA or MDA, while 9 were positive for MDMA. The hair analysis of MDMA showed that a hair sample is a good specimen for the confirmation of long abuse of MDMA and MDA in comparison with urine.

Keywords: MDMA, Hair Analysis, Method Validation

MONITORING DRUG USE THROUGH SEGMENTAL HAIR ANALYSIS. A THREE YEAR CASE STUDY.

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Segmental analysis of hair for the determination of past drug use is an area which receives mixed views within the field of Forensic Toxicology. The aim of this study was to ascertain if an accurate pattern of drug use can be predicted by the segmental analysis of a length of hair. The hair sample (33cm length) was obtained from a 47 year old male with a known history of use of three types of medication. Atenolol (100mg) was taken as required; diazepam (3x 5mg) was taken as required and dihydrocodeine (3 x 30 mg) was taken daily. All three drugs had been prescribed over a period of 9 years following a serious injury.

The hair was cut into eleven 3 cm sections each representing an approximate period of three months (assuming a hair growth rate of 1 cm/month). The segments were prepared for extraction by washing and cutting. Blank hair was obtained from a subject known not to have used any of the drugs under investigation and similarly prepared. Triplicate aliquots (20 mg) of the hair were subjected to overnight acidified methanolic digestion along with standards and blanks. Following digestion the samples were filtered, evaporated to dryness, derivatised using BSTFA 1% TMCS and analysed by GC/MS in the SIM mode.

Analysis of atenolol in the hair segments showed a variable pattern of use. No accurate record of atenolol use was obtained other than an increased use for short periods of time 9 and 18 months prior to sampling. The highest concentration of atenolol in the hair segments was obtained in segment three (corresponding to 6-9 months hair growth). A slight increase in the levels measured in segment six (corresponding to 15-18 months) was also observed.
The analysis of diazepam in hair showed an overall increase of drug in hair from the proximal end to the distal end. It is known that the subject decreased the frequency of diazepam use in the 18 months prior to sampling which is consistent with the decreased concentrations in these hair sections. However there is a threefold increase in the amount of diazepam detected between segment six (15-18 months) and segment 11 (30-33 months). No drug use history of diazepam is available for this period.

Finally, the analysis of dihydrocodeine for which the full drug history was known (90 mg/day for 9 years) yielded extremely interesting results. If no environmental issues (e.g. UV exposure, washing) affect the amount of drug in hair then we would expect the levels in the proximal and distal segments to give the same concentration. However a decrease of approximately 90% in the level of dihydrocodeine detected was observed ($r^2 = 0.77$).

This study demonstrates that hair analysis can be used to detect drugs which were taken several years ago. More work is required to determine the exact relevance of history evaluation through segmental analysis but the results for dihydrocodeine indicate that this is not a futile exercise. The importance of obtaining a full drug use history from subjects participating in studies of this type is highlighted in the analysis of both atenolol and diazepam where gaps in the history meant that a full interpretation of the results could not be carried out.

Keywords: Segmental Analysis, Drug Use History

EVALUATION OF KETAMINE ABUSE USING HAIR ANALYSIS: CONCENTRATION TRENDS IN A SINGAPORE POPULATION

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Ketamine has been used as an anaesthetic since its discovery in 1961. Today, the drug is used primarily in veterinary medicine, and in some short-term surgical procedures in humans. Ketamine also produces post-hypnotic emergence reactions such as prolonged hallucination and delirium, which has led to its abuse. Due to the increasing abuse of ketamine in Singapore, ketamine, norketamine and its dehydro derivatives were listed as Class B Controlled Drugs under the Misuse of Drugs Act (CAP.185) in September 1999. As a drug of abuse, ketamine can be administered orally, snorted, or injected. In Singapore, the most common mode of administration is through snorting. The majority of the ketamine abusers consist of teens and young working adults.

The laboratory has been analyzing ketamine in urine of suspected abusers since September 1999. In the period of January 2000 to April 2004, there were a total of 17,133 urine samples submitted for ketamine analysis, out of which 26.6 % were tested positive for ketamine during the period of January 2000 to April 2001. The number of positive samples increased significantly to 51.2 % during the period of May 2003 to April 2004. Recently, the enforcement agency of Singapore has indicated interest in the detection of ketamine in hair. This is because many of the abusers have claimed that their drinks were spiked with ketamine to avoid prosecution. The detection of ketamine in hair would determine whether they were habitual users or it was an one-off consumption.

This paper presents a method for the detection of ketamine in hair. Hair samples (25 mg) were washed, pulverized and digested in hydrochloric acid (0.5M) overnight at 45°C. The samples were extracted by an automated solid-phase extraction procedure and the extracts were subsequently analyzed using gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring mode (SIM). Good linearity up to 120 ng/mg was obtained for both ketamine and norketamine (r^2 = 0.9987 and r^2 = 0.9985, respectively). Limit of detection (LOD) was found to be at 0.4 ng/mg for both drugs while the limit of quantitation (LOQ) was found to be 0.6 and 0.8 ng/mg for ketamine and norketamine, respectively. Other parameters such as intra- and inter-day variation were also determined.

About 91 hair segments from suspected ketamine abusers were analyzed. In most of the hair segments, ketamine was found to be the predominant analyte (norketamine to ketamine ratio < 1). This is in contrast to urine where the metabolite norketamine is usually found to be the predominant analyte. The range of ketamine detected in hair was found to be from 0.6 ng/mg to 489.0 ng/mg (Mean = 49.0 ng/mg) whereas the range of norketamine detected was from 0.8 ng/mg to 196.3 ng/mg (Mean = 12.1 ng/mg). At concentrations higher than 120 ng/mg, about 5 mg of hair is sufficient for the re-analysis of the sample. Based upon the voluntary confession of the ketamine abusers, a correlation between the amount of ketamine detected and the frequency of abuse was observed. For abusers who snort the drug occasionally (once a week), the concentration of ketamine detected in hair was in the range of 1.1 ng/mg to 42.7 ng/mg (Mean = 9.9 ng/mg). For those who abuse the drug more frequently (twice or thrice a week), the concentration of ketamine detected was in the range of 13.5 ng/mg to 111.1 ng/mg (Mean = 37.4 ng/mg). For those who abuse the drug daily, the concentration of ketamine detected was above 45.1 ng/mg (Mean = 121.3 ng/mg). Based on the results of the analysis, three types of trends for ketamine abuse were observed: Low (1.0 – 10.0 ng/mg), medium (11.0 – 50.0 ng/mg), and high (>50.0 ng/mg). Segmental analysis of the hair samples of the abusers was also performed and its significance is discussed in relation to the history of drug use.

In conclusion, ketamine in hair can be determined by using acid digestion and subsequent GC/MS analysis. Based on the amount of ketamine found in hair, certain patterns of consumption among ketamine abusers can be demonstrated.

Keywords: Ketamine, hair analysis, GC/MS/SIM

THE REQUIREMENT FOR EFFECTIVE WASH PROCEDURES FOR HAIR TESTING, AND THE APPLICATION OF A WASH METHOD TO SAMPLES SOAKED IN COCAINE SOLUTIONS OF 1 TO 50 UG/ML

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It has been proposed that issues of external contamination of hair can be resolved by relying on the presence of metabolites. However, even if some metabolites such as cocaethylene are sufficiently definitive to indicate certainty of use, others such as benzoylecgonine also occur as contaminants. In addition, for phencyclidine only the parent is determined. Therefore effective washing procedures are important in order to rule out contamination as the source of the parent and or metabolite. The protection provided by cutoffs is also dependent on effective washing procedures to remove surface contamination; such contamination can range from very little to at least as much as 20 times the amount in the hair. In testing head hair samples from 75 drug rehabilitation subjects with cocaine-positive urines, cocaine contamination ranged from 0.7 to 8009 ng/10 mg hair; the amounts in the washes ranged from 4% to over 2000% of the drug content of the hair. The wash procedure used for these samples was as follows. First, dry isopropanol (2 mL) was added to about 12 mg of hair in 12 x 75 mm tubes; the tubes were shaken vigorously at 37°C for 15 minutes, after which the isopropanol was removed to a separate tube and saved for later analysis. Then 2 mL of 0.01 M phosphate buffer/0.01% BSA, pH 6, was added to the hair samples in the tubes and the tubes shaken vigorously for 30 minutes at 37°C, after which the buffer was removed and saved to a another tube for later analysis. This 30-minute wash was repeated twice more, followed by two 60-minute washes using the same conditions. After the final (5^{th}) phosphate buffer wash and removal of the buffer, the hair sample was enzymatically digested for confirmation by LC/MS/MS. The washes are routinely analyzed by RIA. The wash criterion is computed by multiplying by 5 the amount of drug per mg hair in the last wash and subtracting the result from the amount of drug per mg hair in the hair digest. The Wash Criterion is actually an overestimate of the amount of drug that would be removed by 5 additional 1-hour washes. If the result after the subtraction is less than the cutoff for the parent drug, the result is considered negative in indicating drug use. The parent-drug cutoff value for cocaine was 5 ng /10 mg hair (500 pg/mg).

The effectiveness of the wash procedure was challenged by contaminating negative hair (including blonde, auburn, brown and black samples) by soaking for one hour in 1000, 10,000, and 50,000 ng/mL cocaine. These amounts far exceed any likely real-life contamination scenarios. After drying, the samples were washed by the procedures described and digested for confirmation by LC/MS/MS The uptake of the cocaine was approximately linear with increasing concentrations of cocaine in the soaking solutions, and the amounts of cocaine in the hair and washes were correspondingly increased. Total cocaine measured in the wash and hair ranged from 20.6 to 66.5 ng cocaine/10 mg hair for those samples soaked in 1000 ng/m; from 185.6 to 590.7 for those samples soaked in 10,000 ng/m; and 885.6 to 1916.7 for those soaked in 50,000 ng/mL. By application of the wash criterion, all samples were clearly identified as contaminated. The values for hair digests after application of the wash criterion were –0.04 to 1.45 ng/10 mg hair for samples soaked in 1000 ng/mL; –18.28 to 2.83 for those soaked at 10,000 ng/ml; and –258.0 to –42.9 for those soaked at 50,000 ng/10 mg hair. Thus the wash procedure and criterion were effective even for extreme conditions of contamination by soaking.

Keywords: Hair, Contamination, Washing

SCREENING OF BENZODIAZEPINES AND HYPNOTICS IN HAIR BY LC-MS/MS: APPLICATION TO FOUR DRUG-FACILITATED CRIMES

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The use of a drug to modify a person's behavior is not a recent phenomenon, but reports dealing with drug-facilitated crimes (sexual assaults, robbery...) are constantly increasing. Among drugs involved in such cases, benzodiazepines (flunitrazepam, bromazépam,...) and hypnotics (zopiclone, zolpidem) are largely concerned.

In most cases and due to a long delay between the event and the claim to the police, blood and even urine samples are of little value. This is the reason why this laboratory developed a original approach based on hair testing. In practice, hairs of the victim are collected about one month after the alleged offense, segmented and analyzed by LC-MS/MS. In positive cases, the drug is supposed to be found only in the segment corresponding to the period of the event.

The Hair strand is twice decontaminated using methylene chloride and then segmented (3 segments of 2 cm). Each segment is cut into small pieces (<1mm). About 20 mg are overnight incubated in phosphate buffer at pH 8.4, in the presence of 1 ng of diazepam-d_5 used as internal standard (IS). After a liquid-liquid extraction with a mixture of methylene chloride/diethyl ether (80/20) and evaporation to dryness, the residue is reconstituted in 50 µL of acetonitrile/water (50/50).

Chromatography is achieved using a XTerra MS C18 column (100 x 2.1 mm, 3.5µm) eluted with a gradient of acetonitrile and formate buffer delivered at a flow rate of 0.2 mL/min.

A Quattro Micro triple-quadrupole mass spectrometer is used for analyses. Ionization is achieved using electrospray in the positive ionization mode (ES+). For each compound, detection is related to two daughter ions.

To demonstrate the applicability of this method, 4 expertises are describe as follows:

1. A-21 year old girl was offered a coffee that made her unconscious. The victim's hair, collected 15 days after the alleged offense, was tested by LC-MS/MS and the first 2 cm segment was positive at a concentration of 4.4 pg/mg whereas the 2 other segments were zolpidem free.
2. A 16-year old girl claimed to have been raped during an afternoon, while sedated. Hair was sampled 9 weeks after the alleged offense and the first 3 cm segment was positive for zopiclone at a concentration of 4.2 pg/mg, the second (3 to 5 cm) at 1.0 pg/mg, whereas the last segment (5 to 7 cm) was zopiclone free.
3. A 39-year old woman felt sleepy for 24 hours after having drunk a coffee. Hair was collected 1 month after the event and the proximal 2 cm-long segment was positive for bromazepam at 10.3 pg/mg and the other segments (2-4 and 4-6 cm) remained negative.
4. A woman was confined illegally for 12 days, repetitively raped and forced to sign cheques, while being under the influence of an hypnotic. Hair was positive for 7-aminoflunitrazepam in the proximal segment (0 to 3 cm) at 31.7 pg/mg, in the second segment (3 to 6 cm) at 2.0 pg/mg and was negative in the distal one (6 to 9 cm).

In drug-facilitated crimes, hair is a very useful alternative specimen, and the concentrations measured, in the pg/mg range, require tandem mass spectrometry.

Keywords: Hair, LC-MS/MS, drug-facilitated crime

QUANTIFICATION OF AMPHETAMINE AND METHAMPHETAMINE ENANTIOMERS IN HAIR SPECIMENS BY GC-MS/NCI

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Introduction: Enantioselective separation of amphetamines is of great importance in clinical and forensic toxicology, as the S-(+)-enantiomers of methamphetamine (MA) and amphetamine (AM) are known to be considerably more stimulant than the R-(–) isomers. The determination of their respective enatiomeric ratios allows the distinction between legal intake and illicit MA and/or AM consumption.

A method describing the chiral separation of AM and MA after derivatisation with S-(–)-heptafluorobutyrylprolyl chloride (S-HFBPCl) and quantification by gas chromatography coupled to mass spectrometry operating in the negative-ion chemical ionization mode (GC-MS/NCI), has already been applied to serum, urine and oral fluid. In the present study the previous method was modified to be suitable for abusers' hair analysis.

Material and methods: Hair specimens were obtained from 11 Korean suspected MA abusers. After digestion with 1 M sodium hydroxide at 100°C during 30 min, followed by solid phase extraction using Cleanscreen ZDAU and derivisation with S-HFBPCl, the enantiomers were quantified by GC-MS/NCI using deuterated internal standards were operated. Extraction yields varied between 88.5 and 100 %, LODs were 10.6 and 2.2 pg/mg hair for MA and AM respectively.

Results: Concentration ranges of 25.2 to 101.4 ng/mg hair for MA and 0.69 to 4.54 ng/mg hair for AM were observed. For 2 specimens, only the S-(+)-enantiomer was detected, for 8 specimens the R/S ratio was < 1 (0.17 - 0.59 for MA and 0.12 – 0.31 for AM), whereas for one specimen a R/S ratio of 10.1 and 1.2 was determined for MA and AM respectively.

Conclusions: Similar to serum, urine and saliva, our results point out the predominance of the S-(+)-enantiomer in hair. In one case the R-(–)-MA concentration was higher than the S-(+)-enantiomer. This may be due to the consumption of prescription drugs either containing R-(–)-MA or biotransformed to R-(–)-MA. The present study shows that hair analysis of amphetamines' enantiomers may be helpful to avoid misinterpretation in the discrimination between prescription drug use and illegal drug consumption.

Keywords: Hair analysis, Enantioselective Separation, Amphetamines

DIAGNOSIS OF CHRONIC ALCOHOL CONSUMPTION. HAIR ANALYSIS FOR ETHYL-GLUCURONIDE

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Alcohol abuse is one of the most serious social problems throughout the world. Ethyl-glucuronide is minor metabolite of ethanol and has proved to be a good alcohol marker. In addition, hair analysis has demonstrated its usefulness in establishing chronic consumption of drugs of abuse. The objective of the present study is to develop and validate a sensitive, precise and specific analytical method for the determination of ethyl-glucuronide in hair samples.

During method development the efficacy of extraction of EtG from hair was compared in four extraction methods: a) methanol; b) methanol:water (1:1); c) water; and d) water:trifluoroacetic acid (9:1). Water was found to be the best extracting solvent, since it provided the highest recoveries, with cleaner extracts. The final method is as follows: about 100 mg of hair are sequentially washed with water and acetone. The decontaminated sample is finely cut with scissors, then the deuterated internal standard (EtG-d_5) and 2 mL of water are added. After sonication for two hours, the sample is maintained at room temperature overnight. Derivatization is performed with PFPA. Derivatives are injected into a GC/MS system in the electronic impact mode. The method shows linearity over the range of concentrations from 0.050 ng/mg to 5 ng/mg. Detection and quantification limits are 0.025 ng/mg and 0.050 ng/mg, respectively. Mean recoveries for the three studied concentrations (low, medium and high) are higher than 87%. The coefficients of variation in intra- and inter-assay precision are always lower than 7%. The method is being routinely applied in our lab for the diagnosis of chronic alcohol consumption. Some examples will be shown. All of them derived from divorce proceedings where chronic alcohol consumption was required by the Court. EtG concentrations ranged from 0.05 to 0.75 ng/mg of hair.

Keywords: Ethyl-glucuronide, Alcohol Markers, Hair Analysis

OPIATE CONCENTRATIONS IN HAIR FROM SUBJECTS IN A CONTROLLED HEROIN-MAINTENANCE PROGRAM AND FROM OPIATE–ASSOCIATED FATALITIES

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Introduction: The objective of this study was the determination of the concentration of opiates (heroin = HER, mono acetyl morphine = MAM, morphine = MOR, codeine = COD, acetyl codeine = AC) in hair, and in particular an investigation of dose-concentration relationships as well as the possibility of using AC as a marker for consumption of illicit heroin in contrast to pharmaceutical heroin.

Methodology: After the controlled i.v. administration of pharmaceutical heroin-HCl (10-1000 mg/d), the concentrations of opiates in head hair were determined (n = 46), using a validated GC-MS method with LOD's between 0.02 and 0.04 ng/mg. In addition, a collective of opiate-associated fatalities was examined (n = 24).

Results: The concentrations obtained in the proximal segment (1 cm) of the patients were between 0.04 and 0.21 ng/mg for HER, for MAM between 0.05 and 5.64 ng/mg and for MOR between 0.03 and 8.37 ng/mg. There was no statistically significant difference in the concentrations in comparison to the opiate fatalities (HER 1.55-5.20 ng/mg, MAM 0.04-30.01 ng/mg, MOR 0.03-11.87 ng/mg in the proximal segments). After controlled heroin administration, a correlation between the dose and the total opiate concentration in the hair was found (r=0.66). When considering a single analyte, the coefficient of correlation increased when plasma half-life increases (r=0.42, r=0.58 and r=0.69 for HER, MAM and MOR). COD and AC were detected in 13.0 % and 10.9 % of the samples of the heroin program, as well as in 33.3 % and 16.7 % in opiate-associated fatalities, respectively.

Discussion: The results confirm the first observations of Kintz et al., who found only limited dose-concentration relationships after HER abuse in hair. The correlation is influenced by the plasma half-lifes of analytes. The lack of differences between obtained opiate concentrations in the hair of participants in a controlled heroin maintenance program and of opiate-associated fatalities does not support the hypothesis that a lack of tolerance can be regarded as a potential cause of death. In addition, the absence of AC also in the majority of the deaths questions its suitability as a characteristic marker of a preceding consumption of illicit heroin.

Keywords: Hair analysis, opiates, heroin maintenance program

EVIDENCE OF ADDICTION BY ANAESTHETISTS AS DOCUMENTED BY HAIR ANALYSIS

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Chemical dependency is a disease that can affect all professions. Among the health care professionals, anesthesiologists represent a specific group. Numerous factors have been proposed to explain the high incidence of drug abuse among anesthesiologists. These include : easy access to potent drugs, particularly narcotics, highly addictive potential of agents with which they are in contact, and easy diversion of these agents since only small doses will initially provide an effect desired by the abuser. Opioids are the drugs of choice for anesthesiologists, and among them fentanyl and sufentanil are the most commonly used. Alcohol, mostly in older anesthesiologists, propofol, ketamine, thiopental and midazolam are also abused. In fact, all but quaternary ammonium drugs can be observed. Symptoms of addiction in the hospital include : unusual changes in behavior, desire to work alone, refuse of lunch relief or breaks, volunteer for extra cases, call, come in early and leave late, frequent restroom breaks, weight loss and pale skin, malpractice, behind on charts, etc. Toxicological investigations are difficult, as the drugs of interest are difficult to test for. In most cases, half-lives of the compounds are short, and the circulating concentrations weak. It is therefore necessary to develop GC/MS/MS or LC/MS procedures to satisfy the criteria of identification and quantitation. In most cases, blood and/or urine analyses are not useful to document impairment, as these specimens are collected at inadequate moments. Hair analysis appears therefore as the unique choice to evidence chronic exposure. Depending the length of the hair shaft, it is possible to establish an historical record, associated to the pattern of drug use, considering a growth rate of about 1 cm/month.

After decontamination with dichloromethane, drugs are extracted from the hair after incubation in pH 8.4 phosphate buffer either by liquid/liquid extraction or headspace technology. Opiates, fentanyl derivatives, benzodiazepines and propofol are analyzed by GC/MS, GC/MS/MS, LC/MS/MS and headspace GC/MS, respectively.

The following cases will be reviewed:

- Case 1: 50-year old anaesthetist, positive for fentanyl (644 pg/mg)
- Case 2: 42-year old anaesthetist, positive for fentanyl (101 pg/mg) and sufentanil (2 pg/mg)
- Case 3: 40-year old anaesthetist, positive for codeine (210 pg/mg), alfentanil (30 pg/mg) and midazolam (160 pg/mg)
- Case 4: 44-year old nurse, found dead, positive for midazolam (760 pg/mg) and propofol (1390 pg/mg)

In these cases, the combination of an alternative specimen (hair) and hyphenated analytical techniques (tandem mass spectrometry) appears as a pre-requisite.

Keywords: Addiction, Anaesthetist, Hair

CLOZAPINE DOSE-HAIR CONCENTRATION RELATIONSHIPS: EVALUATION OF INPATIENT AND OUTPATIENT DATA

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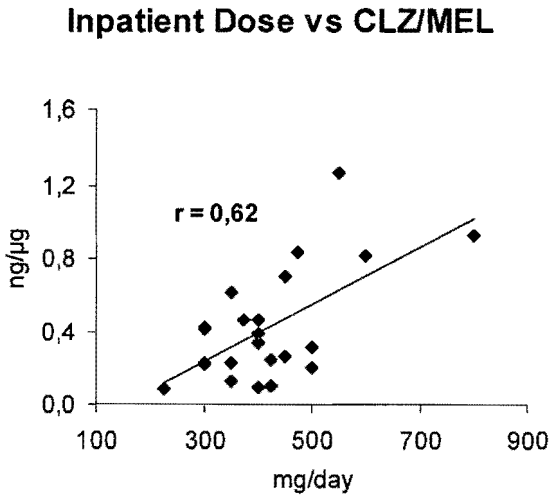
The aim of this work was to test the hypothesis that the concentration of clozapine in hair is dependent on the dose administered. The hypothesis was tested both in an inpatient (N=22) and an outpatient (N=41) population, with and without correction for hair pigmentation.

Samples were taken from the head and stored dark at room temperature until analyzed. Five cm of each hair sample was analyzed. Briefly, the hair was cut into small pieces and weighed into a 10 ml screw-capped tube. To approximately 25 mg of hair were added 0.5 mL of mobile phase (10:10:80 mixture of acetonitrile:methanol:20mM formate buffer pH 3.0) and 25 µl of internal standard (2.0 µg/ml of d3-mianserine) and the sample was incubated in a water bath (with orbital shaking) at 37 °C during 18 hours. A 150-µl aliquot was transferred to an autosampler vial and 10 µl were injected into the chromatographic system.

The LC-MS-MS analysis was performed on a Perkin Elmer Series 200 chromatography system consisting of a Series 200 pump, Series 200 autosampler, and a SCIEX API 2000 MS-MS instrument equipped with an electrospray interface (Turbo Ion Spray). We used a 50×2.1 mm Zorbax SB-Cyano analytical column with 3-µm particle size. For clozapine, two transitions, 327.1/270.0 and 327.1/192.2, were monitored and for d3-mianserine 268.3/208.2 was monitored. All samples were also analyzed for melanin using spectrophotometry (lambda=550 nm) as previously described (Kronstrand et al. Clinical Chemistry 1999, 45:9, pp 1485). The daily doses ranged from 125 to 800 mgs.

The hair melanin concentrations ranged from 5 to 49 µg/mg and the clozapine concentrations ranged between 1.1 to 37 ng/mg. In the outpatient population a coefficient of determination of r=0.14 was achieved when correlating the dose with the hair clozapine concentration, as compared to 0.36 for the inpatient population. When melanin was taken into account the coefficients were increased to 0.36 (out) and 0.62 (in), respectively. The results indicated that melanin influenced the incorporation of clozapine into hair and that the outpatients might have been less compliant than the inpatients, and thus obscuring the results. In summary, it seems that the more controlled the dosing is, and the more factors that influence incorporation are taken into consideration, the better the relationship between dose and hair concentration. Our hypothesis was only confirmed under the certain circumstances of controlled dosing and melanin correction (see figure), otherwise no correlation could be found between the dose and the hair clozapine concentration.



DRUG DETECTION IN ORAL FLUID: IDENTIFICATION OF POLY DRUG USE BY EIA AND GC-MS

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The analysis of drugs of abuse in oral fluid is becoming more widely used and accepted across a number of testing disciplines, particularly because of its simplicity to collect in relation to urine. Commonly abused and prescribed drugs are known to be detectable in oral fluid. The objective of this study was to assess the prevalence of a number of drugs in oral fluid with specimens collected from known and unknown drug users. The cut-off levels for oral fluid are much lower then those for urine. Certain analytical methods that are used with urine samples, including immunoassay and GC-MS, are not necessarily applicable for screening oral fluid specimens.

This study provides a compilation of data for the confirmation by GC-MS/EI, GC-MS/CI or GC-MS/MS of oral fluid for a wide range of analytes. Oral fluid specimens (N=8911) were collected with prior consent from various establishments in the U.K including private companies, rehabilitation clinics, and criminal justice services using the Intercept® DOA collection device (OraSure Technologies, USA) according to manufacturer's instructions. Specimens were initially screened for a combination of Opiates, Cocaine metabolites, Cannabinoids, Amphetamines, Benzodiazepines and Methadone using separate OraSure Technologies Inc. micro-plate enzyme immunoassays and Buprenorphine (Diagnostix Ltd, Canada), according to manufacturer's instructions.

For the confirmation analysis by Gas Chromatography-Mass Spectrometry (GC-MS), the samples were cleaned using Oasis® MCX Cartridges and derivatised with TFA or BSTFA (Sigma, Poole, UK). The derivatised extracts were injected onto either gas-chromatograph HP5973 for GC-MS/EI and GC-MS/CI (Agilent, Berkshire, UK) or Varian Inc. Saturn for GC-MS/MS (Walton-on-Thames, UK) equipped with capillary column 15mx0.25 mm Varian, Factor Four. The dynamic range was from 2 to 2000 ng/mL. Three ions for the drugs and two ions for the internal standards were monitored. The confirmation rate for each drug group, relative to applied cut-off levels, is presented in Table 1.

Table 1. Total oral fluid samples tested by GC-MS for each drug group and confirmation rates.

	GCMS	Confirmation Rate	
Drug Group	**N**	**N**	**%**
Amphetamines	337	305	(91%)
Benzodiazepines	1027	823	(80%)
Buprenorphine	175	104	(59%)
Cannabis	632	316	(50%)
Cocaine	1358	1026	(76%)
Methadone	1536	1437	(94%)
Opiates	6460	5189	(80%)

Percentiles: 50% (Median), 95% and maximum levels for all analytes in each drug group were calculated and will be presented. Detailed results for the Cocaine group of analytes are shown in Table 2.

Table 2. Results showing Percentiles: 50% (Median), 95% and maximum levels in ng/mL for Cocaine group.

	Median	**95%**	**Maximum**	**N**
AEME	62.0	1409.1	3458.9	75
Benzoylecgonine	49.1	566.0	5240.2	865
Cocaethylene	12.3	101.4	136.7	33
Cocaine	30.4	2135.6	29613.4	843

Oral fluid testing offers a convenient, reliable and consistent method to determine the presence of poly-drug use and can identify the presence of specific drug metabolites by the GC-MS methods established.

Keywords: Drugs of Abuse, Oral Fluid, GC-MS

VALIDATION OF THE COZART® MICROPLATE ELISA FOR THE DETECTION OF AMPHETAMINES IN HAIR

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The purpose of this study was to determine the performance characteristics of the Cozart® Amphetamine Microplate ELISA assay for the detection of amphetamines in hair samples. Hair samples (N=50) were collected from volunteers with a history of drug use and from drug related deaths. The hair samples were sonicated in methanol and then extracted overnight at 60°C. The methanol extract was evaporated to dryness and reconstituted in ELISA negative calibrator. 25µL of calibrator (0, 50, 100, 200, 300, 500 and 1000pg/mg), control and sample were assayed in duplicate according to manufacturers instructions for the Cozart® Amphetamine ELISA – Forensic Application Kit.

For GC-MS analysis, deuterated internal standards (amphetamine-d5, methamphetamine-d5, MDA-d5, MDMA-d5, MDEA-d6 and MBDB-d5) mixture, β-glucuronidase and 0.1M, pH 6.0 phosphate buffer were added to approximately 20 mg of sample, or spiked blank hair and sonicated for 1 hour. Isolation of amphetamine, methamphetamine, MDA, MDMA, MDEA and MBDB was achieved through simple solid-phase extraction using Bond Elut certify cartridges.

The true positives (N=20), true negatives (N=30), false positives (N=0) and false negatives (N=0) for different cut-offs with the ELISA were determined by comparison of the ELISA response (normalised to weight of hair extracted) to the GC-MS results as the reference method. Hair concentrations ranged from 0. – 10.1 ng/mg (amphetamine), 0 – 0.8 ng/mg (MDA) and 0 – 17.4 ng/mg (MDMA).

The optimum cut-off for the Cozart® Amphetamine Microplate ELISA was determined to be 500pg amphetamine equivalents/mg hair using a 20 mg hair sample with an overall precision of 100% vs GC-MS.

Keywords: Amphetamines, Hair, ELISA

CANNABINOID ANALYSIS OF ORAL FLUIDS COLLECTED FROM CLINICAL SUBJECTS FOLLOWING ORAL TETRAHYDROCANNABINOL (THC) ADMINISTRATION (HEMP OIL AND DRONABINOL)

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Therapeutic usefulness of oral cannabinoids is being investigated to address analgesia, AIDS-wasting disease, counteracting spasticity of motor diseases, and emesis following chemotherapy. In addition, cannabis is among the most abused psychoactive substances. Following cannabis use, physiological and behavioral changes occur necessitating testing to detect impairment. In recent years, food products (hemp oil) derived from cannabis have been introduced into the U.S. Analysis of hemp oils reveals that THC is present in a wide concentration range. If these products produce positive drug test for urine and alternate matrices, screening programs to identify cannabis use may be affected. A clinical study to investigate the pharmacokinetics and pharmacodynamics of oral THC was performed. The randomized, double blind, placebo-controlled within-subject, inpatient study compared the effects of hemp oil in liquid and capsule form to dronabinol (synthetic THC) doses consistent with therapy for appetite-stimulation. Hemp oil dosing followed manufacturer's recommendations. Five dosing conditions (0.0, 0.39, 0.47, 7.5, and 14.8 mg/day) of oil/capsules were administered three times daily with meals for five consecutive days. One objective was to determine if hemp oil could produce positive cannabinoid alternative matrices tests. Two devices were used to collect oral fluids, OraSure (OraSure Technologies) and Salivette (Sarstedt) from 90-min after the first dose to 1 day after the last dose. Salivette samples were analyzed by LC/MS. Liquid/liquid extractions isolated THCCOOH from 11-OH-THC and THC. Extraction efficiencies were ≥80%. An LC/MSD (Agilent) with an Eclipse C18 column and electrospray interface with data acquired in the NCI mode was used for analysis. SIM ions monitored were: THCCOOH, m/z 343.2; d3-THCCOOH, m/z 346.2; 11-OH-THC, m/z 329.2; d3-11-OH-THC, m/z 332.2; THC, m/z 313.2; and d3-THC m/z 316.2. Limits of quantitation (LOQ) were 1.0, 0.5, and 0.5 ng/mL and limits of detection (LOD) 0.5, 0.2, and 0.2 ng/mL for THCCOOH, 11-OH-THC, and THC, respectively. 511 Salivettes from 7 subjects were analyzed. OraSure oral fluid specimens were analyzed according to manufacturer's specifications with Cannabinoids Intercept™ MICRO-PLATE Enzyme Immunoassay (OraSure Technologies) with a 12 ng/mL cutoff and confirmed by GC/MS/MS. After SPE, extracts were derivatized with HFPA/PFIP. Extraction efficiency was 50%. THC was analyzed on a Saturn Ion Trap Quadrupole (Varian) equipped with a 5% phenyl-methyl column. Ions monitored were: THC, m/z 492 and d3-THC, m/z 495 (parent ions); THC, m/z 238 and d3-THC, m/z 238 (product ions). LOD/LOQ for THC were 0.75 ng/mL. 497 OraSures from 8 subjects were analyzed. All Salivette and OraSure oral fluid specimens were negative at the specified cutoffs. In contrast, several authors reported cannabinoid positive results for Salivette and OraSure oral fluid samples after smoked THC (Samyn, J Forensic Sci, 2002, 47:1380; Niedbala, JAT, 2001, 75:289). In addition, Niedbala found positive tests for OraSure oral fluid samples collected after oral THC (20-25 mg THC-laced brownie), but concentrations were low (≤7 ng/mL). THC is well absorbed, but has low bioavailability (<20%) via the oral route. There is evidence that deposition of THC into oral fluid is via direct sequestering into oral mucosa during drug use with minimal contribution from blood to oral fluid (Hawks, The Cannabinoids, Academic Press, 1983, 8). Characterization of rates of absorption and elimination of orally administered THC with different vehicles (flax oil, sesame seed oil, cookies/brownies, and emulsions) and with direct administration and encapsulation have shown variable absorption of THC. Little data have been published about oral fluid drug levels after oral THC administration; questions remain including the degree of absorption inherent to the collection devices and THC contamination of the oral cavity immediately following hemp oil/capsule administration. The fact that all oral fluid specimens collected were negative with two different devices for THC-containing hemp oils and capsules suggests little contamination of the oral fluid following ingestion of dronabinol or hemp oils by the time the first specimen was collected, at least by 90 minutes after dosing.

Keywords: OraSure, Salivette, Oral Administration of THC

COMPARISON OF ALCOHOL CONCENTRATIONS IN POSTMORTEM BODY FLUIDS

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The determination of postmortem ethanol is probably one of the most important and frequently requested analyses in forensic toxicology. Because of its hydrophilic characteristics, ethanol distributes with body water and studies have demonstrated the existence of substantial site dependence. Factors such as quality and sampling site; trauma to the body; length of time between death and sampling; presence of microorganisms in the body; diffusion of alcoholic beverages present in the stomach into the pericardial fluid; diffusion of ethanol in aspirated vomitus into cardiac blood; blood contamination and unavailability of blood samples must be considered to interpret the postmortem blood ethanol concentration. Due those factors, there is an enormous effort to find alternative sampling sites and correlations between blood alcohol concentration and other biological fluids to establish different procedures for specimens to sample for alcohol analysis.

In addition, considering the medicolegal purposes, the implications of postmortem alcohol concentrations are extremely important, especially when they are found to be above the legal level for intoxication at one specimen and below that level at another.

The use of vitreous humor as an alternative specimen for ethanol analysis in postmortem cases has increased, because it is stable, readily available, easily sampled and less susceptible to bacterial contamination.

In this work it was determinated the ethanol concentrations in biologic fluids, collected during the autopsy examination from 103 cadavers victims of different cause of death, to establish a correlation between the concentrations of ethanol in vitreous humor with urine and blood specimens, sampled from femoral, subclavian and heart.

Determinations of ethanol in the specimens were performed using capillary gas chromatography/flame ionization detector and headspace techniques.

Statistical analysis of the results indicated that there were no significant differences among urine and blood samples, collected in different sites, with vitreous humor.

Comparing the vitreous humor ethanol concentration with respect to Pearson`s correlation test, it was found 0.97 for femoral blood and urine, 0.96 for heart blood, 0.94 for subclavian blood. These results demonstrate that all the fluids tested with vitreous humor were significantly correlated with "p" (associated probability for the used correlation tests) being always lower than 0,05. It indicates that vitreous humor can be use as an alternative sample for urine and blood.

Keywords: Postmortem Specimens, Multisite Sampling, Ethanol.

METHOD DEVELOPMENT OF MICROWAVE-ASSISTED EXTRACTION AND SUPERCRITICAL FLUID EXTRACTION OF SOME PERSISTENT ORGANIC POLLUTANTS IN HUMAN SEBUM

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Sebum has been used to monitor use of controlled drugs as well as exposure to dioxins. Based on this, it is logical to extrapolate its use to also monitor exposure to other environmental pollutants. Methodology is being pursued which will allow the analysis of persistent organic pollutants (POPs) through sebum testing. Statistical Analysis methods based on Minitab® software were applied to optimize microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) conditions for the analysis of some standards representative of POPs. For practical purpose a model matrix of synthetic sebum spiked with the following standards: 1,4- benzodioxan, 1,2,4, 5- tetrachlorobenzene, aldrin, dursban and pyrene was used for the optimization of the extraction techniques. A stock solution of the model matrix was prepared as follow: synthetic sebum was dissolved in dichloromethane and the standards of interest were spiked into the solution. An aliquot 3μL of the spiked sebum solution was taken, deposited on the sebutape® and the solvent allowed to evaporate. The final concentration of the standards, if a 100 % recovery was obtained, was 3 ppm. The Recoveries of analytes were followed using GC-MS. All compounds were successfully extracted from sebum with recoveries ranging from 69 for 1,4 –benzodioxan to 91 % for dursban under the optimum MAE conditions: 5 mL acetonitrile, 70 °C extraction temperature, and 10 min microwave heating. The average recoveries in SFE ranging from 84 for 1,4–benzodioxan to 95 % for pyrene under the following conditions: 30 min of extraction at 40°C, under CO_2 at 4000psi modified with 20% (v/v) methanol. The addition of a small volume (10-20 %) of methanol to the extraction cell enhanced the recoveries of representative persistent organic pollutants. A comparison of MAE, SFE was also conducted. The results indicated that ≥ 69 % average recoveries were obtained by both optimized techniques. Good precision with RSD less than 10% was attained for most of the standards with both techniques. Although with SFE higher percents of recoveries were obtained, it also is more efficient extracting the components of the sebum like saturated hydrocarbons and fatty acids than MAE. In addition to further optimizing our SFE approach, both MAE and SFE are now being compared with ultrasonic extraction commonly used for the extraction of biological matrices. Solid phase extraction, with different stationary phases, was used to remove sebum components from the extract. Also SPME was tested to improve the detection limits. Real samples of human sebum are being analyzed from an area impacted by the emission from an incinerator of biomedical wastes. The compounds found in these persons will be compared to compounds found in air samples from the same region. This will show if specific air pollutants are being incorporated by this population. Once completed and validated this noninvasive approach will provide a powerful method for monitoring exogenous compounds in human.

Keywords: Microwave-Assisted Extraction, Supercritical Fluid Extraction, Sebum

DRUG-FACILITATED CRIMES - INTEREST OF TANDEM MASS SPECTROMETRY AND HAIR ANALYSIS - STATISTICS OF DRUGS IMPLICATED DURING A YEAR OF WORK AT TOXLAB

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AIMS: Drug-facilitated crimes cases are often difficult to resolve, from an analytical point of view, since the drugs administered, largely benzodiazepines or hypnotics, are generally given as one therapeutic dose, and most often have a short half-life elimination. In France, for these kind of aggression cases, the police directs the victim towards a hospital or, in big cities, to an emergency forensic service office. If necessary, after the clinical exam, blood and sometimes urine samples are taken. Using HPLC/DAD and/or GC/MS, immunochemical as well as toxicological screenings are performed on these samples. The immunochemical techniques constantly produce negative results. HPLC/DAD and GC/MS do not permit most basic molecules used in drug-facilitated crimes to appear because the aggression reports, and then the samples, are often late and thus substances are undetectable with those techniques. This is why we have developed an HPLC-MS/MS method for the benzodiazepines and hypnotics determination in biological fluids and hair. By that way, we can reach the detection limits of the ng/mL order in biological fluids and of the pg/mg order in hair. Human hair is sampled by us, about one month after the exposure. The sequential hair analysis by LC-MS/MS allows to affirm that the identified molecules in blood and/or in urine were in fact from an isolated single dose and not from a medical treatment, so it brings evidence for drug-facilitated crimes.

METHODS: The detection of main benzodiazepines in biological fluids were performed by LC-MS/MS after an extraction of 1 mL with Toxitube A® (Varian). Hair was segmented, decontaminated, cut, then 20 mg were extracted with deuterated standard, whether one night incubation in 1mL Soerensen buffer (PH 7.6), or whether, for aminometabolites, at 95° for 15 minutes in NaOH 0,1N. A liquid-liquid extraction was performed with dichloromethane/ether (80/20). The organic phase was filtered before being evaporated. The dry extract was reconstituted by 60µL of ACN/MeOH (50/50) and injected into the LC-MS/MS (ThermoElectron). The liquid chromatography was carried out on an Uptisphere ODB C18 5µ 150 x 2mm column (Interchim) with a gradient of acetonitrile and formate 2mM pH3 buffer. The run was 15 minutes long. The mass tandem TSQ Quantum detection allowed the simultaneous determination of 27 benzodiazepines in SRM mode with two runs.

RESULTS: The detection limits reached by these techniques, are in the order or inferior to 0.5 ng/mL in urine and about 1pg/mg in hair for the 27 benzodiazepines studied. We present two cases of drug-facilitated crimes in which clonazepam and flunitrazepam were found in blood and urine samples. In these two cases, a single dose of these molecules appeared on the specific segment of hair corresponding to the period of the offense. From June 2003 to May 2004 we treated 90 cases of presumed drug-facilitated crimes, for which we had urine and/or hair samples. In 35.5% of these cases, the benzodiazepines or hypnotics were detected. Among these, a single dose, taken without someone's knowledge, appeared in 65.5%. In the 90 cases, 35.5% of the victims were vulnerable following an alcohol and/or illegal drugs consumption ; in 22.2% the investigation showed that it was not a drug-facilitated crimes and 6.7% were not able to be solved.

CONCLUSIONS: This technique produces an analytical response that is essential to police and justice authorities. It brings out criminal acts and produces the scientific proof of drug-facilitated crimes with benzodiazepines and hypnotics as shown by our statistics and the two specific cases cited.

Keywords: Benzodiazepines, LC/MS/MS, Hair Analysis

IMPROVED GCMS ANALYSIS OF THC IN SALIVA

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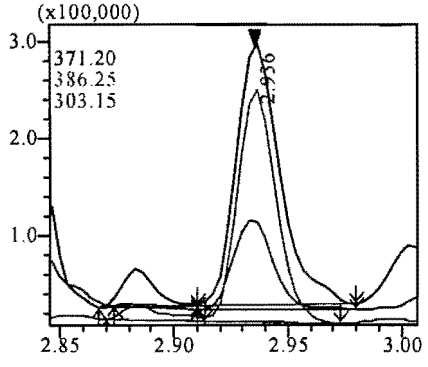
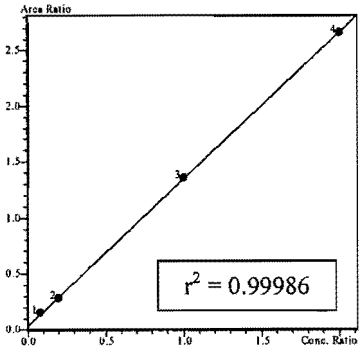
One of the most challenging GCMS applications is the determination of THC in saliva. Detection for THC at low ppb [ng/ml] levels is required due to several factors including; 1) rapid elimination of THC in saliva from time of exposure, 2) low amount of drug for 'active' dose and 3) small sample volume available at time of collection. This application demonstrates an example of how the Shimadzu GCMS QP-2010 can provide the instrument performance necessary to complete this challenging analysis. Samples were collected using the Quantisal ™ saliva collection kit. Extractions are performed by extraction with hexane:ethyl acetate [9:1] with 3 % acetic acid. Derivatization is completed using 50 uL BSTFA to form the trimethylsilane derivative. 1.0 uL of sample extract is injected for analysis. Using the standard SPL-2010 split/splitless injection port on the GC-2010 as a high pressure injector, the method was performed with a splitless injection using a Restek 3.4mm. id. SILTEK liner. With high-pressure injection of 175 kPa, fast temperature ramping of 35 C/min and high column flow rate, the peaks eluted from the column into the mass spectrometer ion source in less than 3.00 minutes. These results were obtained using a 15m X 0.25mm X .25 um phase DB-1MS column. Calibration from 1.0 – 25 ppb THC produced a linearity coefficient of 0.9999 by internal standard calculation using THC-D_3 at 12.5 ppb. Preliminary LOQ is determined to be 0.25 ppb. Precision at 3.0 ppb THC is 5.97%.

1.0 ppb THC [TMS] Standard



Calibration, 1.0 – 25 ppb THC



Keywords: THC, Saliva, GCMS

SIMULTANEOUS ASSAY FOR NICOTINE AND ITS METABOLITES IN ORAL FLUID BY SPE AND GC/MS/EI.

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Nicotine, the major alkaloid in tobacco, is rapidly and extensively metabolized in humans, but its metabolism varies among individuals. Nicotine is primarily metabolized by cytochrome P450 (CYP2A6) to cotinine, which is subsequently hydroxylated to *trans*-3′-hydroxycotinine. This method was designed to simultaneous quantify nicotine, cotinine, norcotinine, and *trans*-3′-hydroxycotinine in human oral fluid, a noninvasive biological matrix. An aliquot (0.5 mL) of each oral fluid sample, quality control (QC) sample, or calibration standard was mixed with 2 mL of 2M sodium acetate buffer (pH 5.5). Deuterated internal standards (d_3-nicotine, d_3-cotinine, and d_3- *trans*-3′-hydroxycotinine) were added to each sample. Solid phase extraction (SPE) columns (200 mg Clean Screen® ZSDAU020, United Chemical Technologies, Bristol, PA) were preconditioned with elution solvent, methanol, water, and buffer. Each sample was loaded onto the SPE column and washed with water, 0.2 N HCl, and methanol. Analytes were eluted with methylene chloride: 2-propanol: ammonium hydroxide (80:20:2 v/v/v), and 100 µL of 1% hydrochloric acid in methanol (v/v) was added prior to evaporation. Extracts were evaporated to dryness under a stream of nitrogen at 40°C using a Zymark Turbovap LV Evaporator. Extracted residues were reconstituted in acetonitrile, derivatized with BSTFA (with 1% TMCS), and analyzed by GC/MS/EI in the selected ion monitoring (SIM) mode. GC/MS analysis was performed using an HP6890 gas chromatograph interfaced with HP5973 mass-selective detector, equipped with HP-5MS column (30m x 0.25mm i.d.; 0.25µm-film thickness) with helium gas at 1.0 mL/min. The instrument was operated in the splitless mode. The initial column temperature of 70°C was held for 1 min, followed by increases to 190°C at 30°C/min, to 230°C at 5°C/min, to 290°C at 25°C/min. The ions for each analyte were monitored in the following elution order (quantitative ions are indicated in parenthesis) for the derivatized analytes: d_3-nicotine, m/z (87), 165; nicotine, m/z (84), 162; d_3-cotinine, m/z (101), 179; cotinine, m/z (98), 176; norcotinine, m/z (234), 219; d_3-*trans*-3′-hydroxycotinine, m/z (252), 147; and *trans*-3′-hydroxycotinine, m/z (249), 144. The base peak and molecular ions of each analyte and internal standards were evaluated by SIM analysis. However, the EI spectra of these drugs do not possess many fragments that can be used as confirming ions. The mid-mass range ions for these drugs contain non-specific interferences probably from co-extracted endogenous material. For this reason, only two ions, the base peak and the molecular ion, were monitored in this assay. Eight point calibration curves for nicotine, cotinine, norcotinine, and *trans*-3′-hydroxycotinine were linear across a concentration range of 2.5 to 500 ng for all compounds/0.5 mL of blank oral fluid. Correlation coefficients of the calibration curves were >0.99. The limits of determination and quantitation are 2.5 ng/0.5 mL (50 pg on column) for all analytes. Recovery was 90 - 115% for nicotine, 76 – 117% for cotinine, 88 –101% for norcotinine, and 66 – 77% for *trans*-3′-hydroxycotinine at 8, 80, and 400 ng/0.5 mL (low, mid, and high, respectively), QC sample concentrations. Intra-assay precision (% CV) and accuracy (percent difference between mean and target concentrations) ranged from 1.6 – 5.7 and 1.6 – 17.8%, respectively, at low, mid, and high QC sample concentrations for all analytes. Inter-assay precision and accuracy for low, mid, and high QC sample concentrations ranged from 4.4 – 8.8 and 0.2 – 12.6%, respectively, for all analytes. Suitable precision and accuracy were achieved for the simultaneous determination of nicotine and three metabolites in the oral fluid of smokers. This assay is applicable to pharmacokinetic studies of nicotine and its metabolites in oral fluid and provides a biomarker for identification of smoking, which could be helpful in smoking cessation programs.

Keywords: Nicotine, Nicotine Metabolites, Oral Fluid

Δ^9-TETRAHYDROCANNABINOL (THC), 11-HYDROXY-Δ^9-TETRAHYDROCANNABINOL (11-OH-THC) AND 11-NOR-9-CARBOXY-Δ^9-TETRAHYDROCANNABINOL (THCCOOH) IN HUMAN PLASMA FOLLOWING ORAL ADMINISTRATION OF HEMP OIL.

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Usefulness of therapeutic oral cannabinoids is being investigated for several medicinal applications. Cannabis is among the most abused psychoactive substances causing behavioral and physiological changes that necessitate testing to detect impairment. In recent years, hemp oil products derived from cannabis have been introduced into the U.S. Analysis of hemp oils reveals that THC is present in a range of concentrations. A clinical study to investigate the pharmacokinetics and pharmacodynamics of oral THC was performed. The randomized, double blind, placebo-controlled within-subject, inpatient study compared the effects of hemp oil in liquid and capsule form to dronabinol (synthetic THC) doses consistent with therapy for appetite-stimulation. The NIDA IRB approved this study and each participant provided informed consent. One aspect of this study was to determine the detection times and levels of THC, 11-OH-THC and THCCOOH in plasma following oral THC administration. Hemp oil dosing followed manufacturer's recommendations. Five healthy volunteers with a history of marijuana abuse ingested commercially available hemp oils/capsules of differing THC concentrations. Five dosing conditions (0.0, 0.39, 0.47, 7.5, and 14.8 mg/day) of oil/capsules were administered three times daily with meals for five consecutive days followed by a ten-day washout period before the next dosing session began. Plasma samples were collected prior to and following each of the five dosing conditions throughout the study and were frozen at -20°C until analysis. Plasma was extracted by SPE (200 mg Clean Screen® ZSDAU020, United Chemical Technologies). The extracts were derivatized with BSTFA (1%TMCS), separated and quantified on a GC/MS (Agilent) operated in the positive chemical ionization mode with SIM monitoring. The following table lists the mean minimum and maximum concentrations (ng/mL) for all plasmas collected from 30 minutes before the first dose to 2 days after the last dose for each session for five subjects:

		THC		11-OH-THC		THCCOOH	
Session	Dose Amount	Min	Max	Min	Max	Min	Max
High Potency Hemp Oil	14.8 mg/day	0.0	6.5	0.0	5.6	0.0	15.2
Dronabinol Capsules	7.5 mg/day	0.0	3.6	0.0	2.6	0.0	24.4
Hemp Oil Capsules	0.47 mg/day	0.0	0.0	0.0	0.0	0.0	2.5
Low Potency Hemp Oil	0.39 mg/day	0.0	1.2	0.0	3.8	0.0	3.1
Placebo	0.0 mg/day	0.0	0.0	0.0	0.0	0.0	0.0

Drug levels of plasmas varied within the same dose between subjects. In general, the THC, 11-OH-THC, and THCCOOH levels were low and erratic across the collection timeline after all doses. The peak concentrations and time to peak concentrations varied, sometimes considerably, between subjects. In general, the maximum concentrations of 11-OH-THC were seen in the liquid hemp oil sessions. The sessions in which capsules were administered had lower concentrations, less than the low dose of hemp oil administered, or zero concentrations. Plasma THC and 11-OH-THC were negative for all participants and for all doses by 16 hours after the last THC dose. Plasma THCCOOH persisted for a longer period of time following the two highest doses of 7.5 mg/day dronabinol and 14.8 mg/day THC in hemp oil. Ohlsson et al. (Clin. Pharmacol. Ther., 1980, 28:409) reported that orally administered (20 mg cookie) THC yielded low and irregular plasma concentrations compared to intravenous and inhaled THC. Bioavailability of orally administered THC is low. This may be due to poor absorption, degradation by stomach acid, or biotransformation to metabolites via first pass through the liver.

Keywords: Oral Administration of THC, Plasma, GC/MS.

A MICROTITER PLATE ELISA PLATFORM FOR SCREENING ABUSE DRUGS IN ORAL FLUID

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The purpose of this study was to evaluate the suitability of microtiter plate ELISA assays (Neogen Corp) for screening of abuse drugs in oral fluid (OF), and the efficiency of drug recovery from a oral sampling device, Sarstedt Salivette®. The following drugs were tested at the proposed cutoff levels for the respective compound according to the Federal Workplace Drug Testing Program draft guidelines: cocaine, morphine, 6-monoacetylmorphine (6-MAM), d-amphetamine, d-methamphetamine, and methylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA). Twenty, non-smoking, drug-free donors by testimony spit approximately 25 mL OF into a clean cup and the sample was then poured away from the froth and centrifuged. All OF samples were confirmed negative by GC/MS for all of the tested analytes. The samples were then pooled to create a blank OF matrix pool used in all assays. The OF pool portions were spiked with the above analytes at the proposed cutoff levels for the respective compound. Next, spiked or blank OF was collected from a cup using an oral sampling device. The collector pad was held with tweezers and immersed in the cups of OF and then processed according to the manufacturer's protocol. OF samples of 20µL were analyzed alongside a standard curve by ELISA to determine the dilution factor needed to bring the $\%B/B_0$ of each drug in OF at the standard proposed industry cutoff levels into the optimal range for the ELISA. The calibration curve was established by plotting log concentration VS logit transformation of the absorbance ratio for each calibrator and the blank sample ($\%B/B_0$). The concentrations of the analyte in the test samples were then determined from the calibration curve based on their absorbance ratios, B/B_0. The percentage of drug recovery from the oral sampling device was calculated by comparing the concentration of the processed sample using the device to the non-processed sample concentration. The amount of drug detected by the ELISA when no collection device was utilized was determined by Observed/Experimental x 100. The intra-assay precision was evaluated based on the mean absorbance ratio B/B_0 of the 16 replicate analyses of blank samples that were used in LOD determination. These results are summarized in Table 1. The results indicated that this ELISA platform is suitable for the analysis of the following drugs in OF: cocaine, morphine, 6-MAM, d-amphetamine, d-methamphetamine, and MDMA.

Table 1.

Drug	Dilution of OF	Drug Conc. in OF (ng/mL)	Amount Recovered of OF (ng/mL)		Intra-assay Precision of B/B_0
			No Collector	Collector	
Cocaine	1: 50	5 20	4.76±0.15 24.01±1.90	2.07±0.32 14.96±2.47	3.863
Amphetamine	1: 5	25 50	17.86±2.56 40.86±3.85	13.84±0.55 36.55±1.94	3.863
MDA	No dil.	50 100	10.42±2.58 23.58±23.52	56.24±8.96 63.19±16.74	10.581
Methamphetamine	1: 5	25 50	36.37±2.56 56.32±3.20	36.30±10.18 58.42±2.12	2.755
MDMA	1: 5	25 50	30.26±6.45 50.07±5.78	22.53±7.5 44.08±6.64	8.316
Morphine	1: 50	20 40	19.62±5.71 42.36±0.96	15.18±4.43 32.79±7.43	3.798
6-MAM	1: 5	2 4	3.13±0.77 5.67±0.72	2.43±0.19 5.56±1.25	4.564

Keywords: ELISA, Oral Fluid, Drugs of Abuse

LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION MASS SPECTROMETRY FOR THE DETERMINATION OF SELECTED BENZODIAZEPINES

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A simple, rapid, and sensitive method based on the use of liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS), which allows the simultaneous determination of nine Benzodiazepines (Midazolam, Bromazepam, Tetrazepam, Alprazolam, Lorazepam, Triazolam, Diazepam, Flunitrazepam and Lormetazepam) was developed. The method was applied to plasma and saliva samples. The collection of saliva samples were done with the Salivette®device. Lorazepam-d4, Diazepam-d5, and Alprazolam-d5 were used as internal standards.

The separation of the nine benzodiazepines was performed by using acetonitrile/0.1% formic acid in gradient mode as a mobile phase, on a XTerra RP_{18} 5 μm (150×2.1mm I.D.) reverse phase column. The liquid-liquid extraction procedure of the compounds and their respective internal standards was carried out with diethyl ether. The quantification study was done by operating in selected ion monitoring (SIM) mode, and two m/z ratios for each compound were chosen. The method was validated for both biological samples.

	LOD		LOQ		Selected
	Plasma	Saliva	Plasma	Saliva	m/z ratios[a]
Midazolam	0.5	0.1	1	0.2	**326.1** 291.3
Bromazepam	1	0.2	5	0.5	**318.0** 290.0
Tetrazepam	0.5	0.1	1	0.2	**289.2** 261.3
Alprazolam	0.5	0.2	1	0.5	**309.1** 281.2
Lorazepam	0.5	0.2	1	0.5	**321.0** 303.1
Triazolam	0.5	0.1	2	0.2	**343.1** 308.1
Flunitrazepam	0.5	0.1	1	0.2	**314.2** 268.2
Diazepam	0.5	0.1	1	0.2	**285.3** 257.2
Lormetazepam	0.5	0.1	1	0.2	**335.0** 289.0
Lorazepam-d_4	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	325.1
Alprazolam-d_5	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	314.2
Diazepam-d_5	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	290.3

[a]Quantifying ions are in bold characters
[b]N.D.=Not Detected

In addition, a preliminary pharmacokinetic study for two of the nine Benzodiazepines (Midazolam and Lormetazepam) was carried out in order to check the possible saliva and plasma levels correlations.

Keywords: Benzodiazepines, Plasma and Saliva, LC-ESI-MS

DETECTION OF SMOKED COCAINE MARKER (ANHYDROECGONINE METHYLESTER) IN NAILS.

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Objective: The chronic intoxication by smoked cocaine or crack results in psychic disturbances that may lead to asocial and aggressive behavior. For medical, legal and epidemiologic purpose it is interesting to be able to detect this drug-addiction. The detection of anhydroecgonine methy ester (AEME) marker of cocaïne pyrolysis in hair is significant of chronic consumption. The collection of hair is not always possible among Afro-Americans, and that of the nails can represent an interesting alternative to search AEME.

Materials and methods: Two samples of 50 mg of nails collected with informed consent from two subjects consuming smoked cocaine were analyzed by gas chromatography coupled to mass spectrometry detection. The AEME was searched in these samples.

Prior to analysis, the samples were washed with hot water then by 5 baths of dichlorométhane in order to eliminate any possibility of contamination by the contact with the product or the smoke before being analyzed. The analysis was carried out in mode SIM on a Gaz Chromatograph (6890) coupled to a mass selective detector (5973) Agilent Technologies, (Palo Alto, CA) (1). AEME was quantitated by comparing ratios of peak areas with those of deuterated cocaine.

Results: The analysis of the nails samples of both subjects (1 and 2) leads to detection of cocaine, its metabolites and the AEME. The analysis of dichloromethane baths were negative from the 3 me bath.

ng/mg	COC	BE	EME	CE	AEME
Subject 1	28.7	7.3	6.3	< LOD	0.39
Subject 2	34.5	17.9	2.5	< LOD	0.24

Other alkaloids were also detected in nails after washings like the tropacocaine, the pseudocaine and the cinnamoylcocaine indicating the consumption of badly purified cocaine.

Discussion and conclusion: This is, to our knowledge, the first description of the specific marker of smoked cocaine in nails. The precautions of treatment of the sample avoided the risk of external contamination main source of error for this analysis. This result shows that nail specimen is an alternative to hair specimen to display crack cocaïne chronic consumption. Afro-Americains subjects, except the " Rastafari ", often wear very short hair or are shaven but, for cultural reasons, wear long nails. Therefore, detection of AEME in nail specimen seems an appropriate method for this population.

Reference:
1- Pascal Kintz, Catherine Sengler, Vincent Cirimele and Patrice Mangin. Evidence of crack use by anhydroecgonine methylester identification. Human and Experimental Toxicology (1997) 16,123-127.

Keywords: Cocaïne, Crack, AEME, Nails.

DETERMINATION OF THC IN ORAL FLUID SAMPLES USING LC-MS

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Analysis of THC and its metabolites in biological samples is of great relevance for forensic purposes. In the case of oral fluid the analysis should determine THC, whereas in urine, it detects the inactive metabolite THC-COOH. Most laboratories analyze THC in such samples using GC-MS methods, but these procedures are time-consuming and involve unavoidable previous extraction and derivatization. In this paper we report a validation method in which the THC is isolated from oral fluid by a simple liquid-liquid extraction with hexane. The separation and quantitation was done on a positive ion electrospray (ESI positive) high performance liquid chromatography-mass spectrometry (LC-MS) in the selected ion monitoring (SIR) mode. Calibration curves for Δ^9-THC were performed in oral fluid, achieving linearity between 2 ng/mL and 100 ng/mL. The procedure was validated in terms de linearity (coefficient of correlation: 0,9956±0,0035), repeatability (5.33-10.02 %, n=6), reproducibility (2.58-14.7 %, n=6). The limit of quantification (LOQ) was defined as the lowest concentration of analyte that could be measured reproducibly and accurately (CV < 20% and bias > 80%), which was 2 ng/mL. The LOD was estimated on the criteria of the lowest concentration of an analyte that the bioanalytical procedure can reliably differenciate from the backgroud noise, which was 1 ng/mL. The mean recovery was 86.62%. The method requires only 200 µL of oral fluid, and has been used to analyze oral fluid samples collected from drivers, by spitting, salivette and intercept® modes.

Keywords: LC-MS, Oral Fluid, THC

DOSE-RELATED DISTRIBUTION OF CODEINE, COCAINE AND METABOLITES INTO HUMAN HAIR FOLLOWING CONTROLLED ORAL CODEINE AND SUBCUTANEOUS COCAINE ADMINISTRATION

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Ten volunteers with histories of cocaine and opiate use consented to reside on a secure clinical research ward at the Intramural Research Program, National Institutes on Drug Abuse, National Institutes of Health to participate in a ten-week controlled cocaine and opiate administration study. This Intramural Research Board approved study was designed to investigate excretion of opiates, cocaine and metabolites into hair following multiple subcutaneous (s.c.) cocaine hydrochloride and oral (p.o.) codeine sulfate doses. The first three weeks of the study comprised the drug washout phase. During the low dose drug administration week (week 4) 75 mg/ 70 kg cocaine, s.c. and 60 mg/ 70 kg codeine, p.o. doses were administered on alternating days for a total of three doses for each drug. The high dose drug administration week began on week 8 during which, subjects received three doses of 150 mg/ 70 kg cocaine, s.c. and 120 mg/ 70 kg codeine, p.o. on alternating days. An electric razor was used to collect hair at the end of each week during the study. Drugs were extracted from pulverized hair via methanolic sonication and extracts were analyzed for cocaine, norcocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), cocaethylene (CE), codeine, norcodeine, morphine and 6-acetylmorphine (6-AM) via liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry. Limits of quantitation (LOQ) for cocaine and metabolites were 17 pg/mg, except for EME (50 pg/mg). Opiate LOQs were 83 pg/mg, except for norcodeine (240 pg/mg).

Maximum cocaine and metabolite concentrations (Cmax) were generally measured in hair samples collected two weeks after drug dosing (range: one to three weeks). Average cocaine and metabolite Cmax's (± standard error of the mean) measured in hair reflecting the low dose cocaine week administrations were 2,997 ± 619, 314 ± 51, 187 ± 27, 84 ± 21 pg/mg of cocaine, BE, EME and norcocaine, respectively. Mean cocaine and metabolite Cmax's measured reflecting the high dose cocaine week administrations were 6,419 ± 1,698, 708 ± 163, 425 ± 65, 218 ± 46 pg/mg of cocaine, BE, EME, and norcocaine, respectively. Norcocaine did not exceed the LOQ after the low cocaine dosing in one of ten individuals. In all other cases, cocaine and metabolites exceeded the LOQ after low and high cocaine dosings. Comparison of cocaine and metabolite hair Cmax's that occurred after low and high dose cocaine weeks revealed significant dose-relationships and were significantly different from hair concentrations at the conclusion of the washout phase as evaluated using Wilcoxon signed rank test ($p<0.05$).

Maximum codeine concentrations also were measured in hair samples collected two weeks after drug dosing (range: one to three weeks). The average codeine Cmax (± standard error of the mean) measured in hair reflecting the low dose week administrations was 1,291 ± 182 pg/mg. Similarly, the mean codeine Cmax after the high dose week administrations was 2,725 ± 476 pg/mg. Norcodeine was present in five of ten subjects' hair following high codeine dosing (range: 250 – 493 pg/mg). Morphine and 6-AM were not present in any samples following dosing. Comparison of codeine hair Cmax's following the low and high dose administrations using the Wilcoxon signed rank test revealed a significant difference ($p<0.05$). No samples contained codeine concentrations greater than the LOQ at the conclusion of the washout phase.

In conclusion, this work demonstrates dose-related concentrations of codeine, cocaine and metabolites in hair following controlled administration of cocaine and codeine.

Keywords: Cocaine, Hair, Alternative Matrices

CODEINE AND METABOLITE DISPOSITION IN HUMAN SWEAT FOLLOWING CONTROLLED ORAL CODEINE ADMINISTRATION

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Analysis of codeine is included in drug treatment, military, criminal justice and workplace programs because of its abuse potential and performance impairing effects. Interest in monitoring drug exposure with human sweat, as an alternative biological specimen is increasing, as sweat collections are convenient, less invasive and difficult to adulterate. Our objective for this study was to determine the excretion profile of codeine and metabolites into human sweat using the PharmChek™ Sweat Patch. Volunteers with a history of opiate use (n=9) provided informed consent to participate in this IRB-approved study and resided on the closed clinical research ward for 12 weeks. Low (60mg/70kg) and high (120mg/70kg) doses of codeine sulphate were administered three times a week, four weeks apart. Codeine, norcodeine, morphine, normorphine, and 6-monoacetylmorphine were isolated by SPE and GC/EI-MS. The limit of quantitation (LOQ) for codeine and morphine were 2.5 ng/patch, and 5 ng/patch for metabolites.

We present data from patches representing four categories of sweat collection. First, weekly sweat patches were collected from the time of entry onto the secure ward until the time of first codeine administration, reflecting excretion of previously self-administered opiates. No patch was positive for 6-acetylmorphine, codeine, morphine or metabolites at the methods' LOQs.

Second, weekly sweat patches also were applied prior to and removed 7 days after dosing for 5 of 9 and 3 of 6 participants for the low and high doses, respectively. 80% of the low and 100% of the high dose weekly patches were positive for codeine, but negative for metabolites. The mean codeine concentration for these patches was 65 ng/patch (median 27, SD 92, range 16-225) for the low dose and 82 ng/patch (median 84, SD 15, range 67-96) for the high dose.

Third, other sweat patches were worn for between 1 to 15 hours during the first 48 hours after dosing. Of 139 patches worn during the first 48 hours following the first codeine administration, only 11 (8%) contained codeine levels greater than our LOQ. Codeine was the only analyte detected and could be found as early as 1 hour after dosing. A total of 7 patches (8%) were positive for codeine following the low dose, and 4 patches (8%) following the high dose. The mean codeine concentration (data from 3 of 9 subjects) in sweat was 43 ng/patch (median 28, SD 50, range 5 - 153) for the low dose and 12 ng/patch (median 11.0, SD 9.0, range 3-24, data from 3 of 6 subjects) for the high dose. Codeine was detected in sweat patches for up to 26 h after administration; patches applied after 26 hours and worn for up to 15 hours were below the LOQ.

Fourth, weekly patches also were applied to 4 of 9 participants two weeks after the last codeine dose. All of the patches also were negative for codeine and metabolites.

Sweat has recently been proposed by the Substance Abuse and Mental Health Services Administration (SAMHSA) for federally-mandated workplace drug testing. A confirmation cutoff of 25 ng/patch has been proposed for patches worn 7 to 14 days. Two patches for one subject and 1 patch for another collected within the first 48 hours following dosing, would be positive using these criteria. In comparison, 6 of 8 patches worn for 7 days during the dosing period would be positive under the new SAMHSA guidelines. Sweat provides an adequate matrix for detection of opioid use. There was large intra- and inter-subject variability. No correlation of codeine concentration with dose was found for the doses investigated. Patches worn for 1 week were more likely to contain codeine above the LOQ than patches worn for 1 to 15 hours.

Keywords: Sweat, Codeine, GCMS

DOPING STEROID ANALYSIS IN NAIL CLIPPINGS

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Extraction of analytes from fingernail is difficult due to its hard keratin composition. Our group has shown the feasibility of drug analysis in nail using a cryogenic grinding technique, including cannabinoids and opioids (1). In particular, cryogenic grinding has been shown to allow the extraction and analysis of intact esters such as diamorphine by avoiding the alternative alkaline hydrolysis step. To date, few papers have reported the detection of steroids in nail (2). By contrast, several papers have reported the detection and analysis of anabolic steroids in hair.

In the present study, cryogenic grinding was used to prepare nail clippings from doping abusers for extraction of endogenous and anabolic steroids to show that both can be detected, as in hair. This method was compared to blood (plasma) and urine samples that had been analysed at the same time.

Fingernail clippings from users of anabolic steroids (including testosterone esters, stanozolol and methenolone acetate) were obtained from the Institute of Doping Analysis/Sports Biochemistry, Kreischa. Blank nail samples were obtained from volunteers. The nail samples were first decontaminated by washing with 0.1% sodium dodecyl sulfate, water (x 3) and methanol (x 3). After drying, the samples were pulverised for 4 min in a liquid-nitrogen cryogenic mill (SPEX CertiPrep 6750 Freezer Mill). The powdered nail was then extracted with methanol/ethyl acetate (7:3 v/v, 7 mL). Deuterated anabolic steroids (5a-estran-3ß-ol-17-one-d_3, testosterone-d_3, and stanozolol-d_3) and medroxyprogesterone were used as internal standards.

The extracts were converted to TMSi derivatives with MSTFA/NH_4I/2-mercaptoethanol and analyzed by GC-MS in the EI + full scan and SIM modes, on a Finnigan Trace instrument equipped with an HP-5 column (30 m x 0.32 mm i.d.; film thickness 0.25 µm) with temperature programming from 180 °C to 240 °C at 3 °C/min, 240 °C to 300 °C at 5 °C/min, and final temperature held for 10 min.

In this initial study endogenous steroids (androsterone (And), etiocholanolone (Etio), dehydroepiandrosterone (DHEA), epiandrosterone (epi-And), epitestosterone (epi-Test) and testosterone (Test)) were identified and quantified in the nail samples from both steroid users and non-users.

No.	And	Etio	DHEA	epi-And	epi-Test	Test
Suspect #1(Nail)	4.1	0.0	5.6	3.3	3.0	4.3
Suspect #2(Nail)	4.7	0.0	3.0	1.9	1.5	3.9
Suspect #3(Nail)	9.6	0.0	25.3	14.3	11.0	10.6
Suspect #4(Nail)	12.8	0.0	12.0	6.2	10.9	25.0
Suspect #5(Nail)	1.7	0.0	1.8	0.8	0.6	0.6
Healthy #1(Nail)	1.5	0.0	1.4	0.7	0.6	6.5
Healthy #2(Nail)	8.4	0.0	4.5	1.7	2.6	1.1
Healthy #1(Plasma)	30322.9	54222.5	11218.3	Trace	222.2	3583.8
Healthy #2(Plasma)	9923.3	2966.7	100.6	Trace	200.2	196.0
Healthy #1(Urine)	351.6	392.5	11.2	Trace	9.0	11.0
Healthy #2(Urine)	926.8	868.7	115.8	Trace	7.4	13.6

* Suspect #3
Test 292 pg/mL in urine, Test-enanthate, decanoate in hair were 20 and 18 pg/mg, respectively.

* Suspect #4
Test 30 pg/mL in urine, Test-enanthate, phenyl-propionate, decanoate, and isocaproate in hair were 25, 425, 220, and 340 pg/mg, respectively.

In general, endogenous steroid concentrations in nail were low, in the pg/mg range. Also, elevated concentrations of testosterone in nail were positively associated with high concentrations in plasma and urine. However, although the analytical results provided evidence for the presence of anabolic steroids in the samples from steroid users, including testosterone and testosterone esters at low concentrations, it has not yet been possible to confirm this due to interference from other endogenous substances. Nail remains a potential, but still to be confirmed, alternative biological specimen to hair for the detection of past exposure to doping steroids.

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Keywords: Nail Analysis, Doping, Anabolic Steroids

DETERMINATION OF Δ^9-TETRAHYDROCANNABINOL (THC) IN ORAL FLUID USING SOLID PHASE EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Aim: High-performance liquid chromatography with electrospray ionization mass spectrometry was used to determine Δ^9-tetrahydrocannabinol (Δ^9-THC) in oral fluid.

Methods: Oral fluid samples were extracted using Bond Elut LRC-Certify solid-phase extraction columns (10cc, 300mg), elution performed with n-hexane/ethyl acetate. The separation and quantitation was done on a positive ion electrospray (ESI positive) high performance liquid chromatography-mass spectrometry (LC-MS) in the single ion recording (SIR) mode. Quantitation was achieved by the addition of the deuterated analogue as internal standard. The compounds were detected by single ion monitoring of m/z 315 and m/z 318 for the protonated molecules [THC+H^+] and [d_3-THC+H^+], respectively. In the chromatographic separation, a X-Terra™ MS C_{18} column (2.1x50mm, 3.5µm) was used and the mobile phase was composed of acetonitrile and ammonia 0.05%, at a 0.3 mL/min flow rate.

Results: No interferences were detected in 10 blank oral fluid samples of different origin. The precision and accuracy were tested on spiked oral fluid samples at three different concentrations (2, 25 and 100ng/mL). The mean recovery was 79%, coefficients of variations were between 2.9-6.9% and the limits of detection (LOD) and quantitation (LOQ) were 1.0 ng/mL and 2.0 ng/mL, respectively. Calibration curves for Δ^9-THC were performed in methanol solutions and in oral fluid samples using seven different concentrations, achieving linearity between 2 ng/mL and 100 ng/mL. This validated method is currently being applied to real cases where oral fluid specimens are collected with the Salivette® and also by spitting from marijuana smokers attending the world's largest music event in recent years, "Rock in Rio – Lisboa".

Conclusions: The method is sensitive, accurate and reproducible and may be utilized in ongoing controlled cannabinoid administration studies and in roadside studies and thus, important for the fields of forensic toxicology.

Keywords: Δ^9-THC, Oral Fluid, LC-MS

LEGAL ISSUES OF ADDICTION AND HAIR TESTING FOR DRUGS OF ABUSE

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Aim: The purpose of this paper is to present legal issues of addiction and hair testing results regarding to the service of Law in Greece.

Methods – Legal Part: Details (such as the process, the decision and the competence of the Court, the police record, the indictment, the expert reports, the defendant's individuality, the crimes and the penal confrontal and many others) from legal cases related to addiction and its judicial verification were collected and analysed.

Methods – Laboratory Part: Laboratory data of cases concerning the laboratory evaluation of addiction in drug users and also occasionally the legal course of cases with addict defendants are presented. In four representative cases segmental hair analysis proved that as long as the individuals were imprisoned findings with drug substances corresponding to that period were lesser or practically absent comparably with samples corresponding to the time out of prison, which proved increased drug abuse.

Results: Hair analysis provides information on chronic exposure rather than acute poisoning. Its detection window varies from some days to months or even years and the length of the hair is the only limit. The procedure that the Law lays in many cases is insufficient and in most cases unable to be abided by. Details about the addicted person's profile are presented. Our study presents that the segmental hair analysis method is a subject of growing interest for drugs of abuse because it provides information on chronic exposure to the drug and the drug history profile of each subject.

Conclusions: Although hair testing for drugs of abuse is not specially referred by any law, Greek Courts accept the method for the final conclusion about the defendant's claim of addiction. Segmental hair analysis may be the only tool to prove this claim, not only when the medical examiner is not able to decide about defendant's addiction and it's severity but also in cases where the time period of drug abuse is to be defined.

Keywords: Addiction, Legal and Judicial Issues, Segmental Hair Testing.

ENHANCED SENSITIVITY FOR DRUGS OF ABUSE IN ORAL FLUID USING THE INNOVATIVE NEW ORALSTAT TEST SYSTEM FOR ON-SITE TESTING.

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The New OralStat is an innovative test system for the rapid on-site detection of drugs of abuse in oral fluid that dramatically improves the limits of detection. This test system consists of an untreated collector sponge with a built-in sample adequacy feature, an integrated test device containing separate receptacles for a sample buffer, gold conjugates, and immunochromatographic test strips. The device allows the lateral flow process to be separated into discrete stages by enabling the dilution of the oral fluid sample with buffer, the preincubation of the sample with the antibody-coated gold reagents, then allowing the sample mixture to chromatograph on the lateral flow test strips. The use of a buffer minimizes the problems associated with viscous saliva. The preincubation of the sample with the antibody-coated gold reagent greatly improves sensitivity. The device can accommodate any combination of 6 drug assays.

The following performance was observed in a study involving spiked oral fluid from 3 non-users:

Drug	Sensitivity (ng/mL)
THC (parent compound)	25
PCP	5
Amphetamine	25
Methamphetamine	25
Opiates	20
Cocaine (Benzoyl Ecgonine)	12
Benzodiazapines	25
Methadone	25

The New OralStat test system can detect PCP, Opiates, Amphetamine, Methamphetamine, and Cocaine metabolite at lower levels than the SAMHSA proposed cutoffs. THC detectability at 25 ng/mL, though still above the SAMHSA cutoff of 4 ng/mL, is a major improvement over current POCTs, improving the detection of recent marijuana use by many hours. The results of a current study with samples from drug users will be presented.

The New OralStat test system is visually read and requires no instrumentation to attain these levels of sensitivity. It is however expected that the use of a reflectance reader along with agitation during the preincubation period will further improve these limits of detection. Initial results show that by applying such measures the THC cutoff of 4 ng/mL can be achieved.

Keywords: Oral Fluid, Drugs of Abuse, On-Site Testing

COMPARISON OF ORAL FLUID WITH URINE TESTING IN DRE CERTIFICATIONS

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Determination of impairment by drugs in DWI cases or in the work place often requires the analysis of biological specimens. The most common matrices tested are blood, urine and saliva. Each of these matrices has inherent advantages and disadvantages. For example, urine has advantages of providing a historical perspective to drug use yet does not supply impairment interpretation. Saliva has the potential for supplying interpretation with respect to blood concentration and impairment with similar detection widows to blood. This abstract reviewed 61 DRE cases, July 8th 2002 until June 30th 2003, involving roadside stops for DWI and DRE certifications events. Twenty-six of the 61 cases involved DRE certification events such as; concerts, and evaluation nights arranged by various police departments were the subject of this initial study. During the certification events the laboratory personnel was able to attend and assist law enforcement with the collection of oral fluids and urine samples. Oral fluids were collected using the Intercept Collection device from OraSure Technologies Inc. The process entailed having the subject place an oral collection pad (attached to a handle) between the lower cheek and gum, rubbing back and forth until moist. Most subjects just sucked on the pad until the sampling time interval was completed (2 minutes). The pad was placed in the sampling tube containing 800 uls of OraSure buffer solution. The specimens were collected typically upon completion of the DRE evaluation. Matched urine and oral fluids specimens were collected. The oral fluids were tested in the OTI Intercept® Micro-Plate EIA, while the urines used the OTI Micro-Plate EIA – serum kits optimized for urine analysis. Both analyses were performed on a PersonalLAB™ automated analyzer. All subjects were screened for seven classes of drugs: Benzodiazepines, Cocaine, Cannabinoids, Opiates, Methamphetamines, Amphetamines, Barbiturates, PCP, and Methadone.

Summarized in Table 1 are the correlated analytical results between urine and oral fluid testing as they relate to DRE interpretation. The positive rates with respect to the various drug categories appears to be the highest for Cocaines and Cannabinoids, 87% and 94% respectively, while Barbiturates had the highest percentage of oral fluid positive results 75% versus 25% for urine. DRE percent corroboration varied between 25% for Stimulants, 36% for Depressants, 47% for Cannabinoids, and 100% for Narcotic Analgesics. These results seem to parallel with the subject's admission rate. Certainly one issue confounding the DRE corroboration was the percentage of positive polydrug cases. 57% of the cases had two or more drug categories test positive, 27% screened positive for three or more drugs, 15% of the cases screened positive for four or more drugs, and 8% of the cases screened positive for five or more drugs.

The ease of administration for oral fluid testing in DRE evaluations may provide additional information with respect to impairment interpretation. This initial study demonstrated that Cocaine and Cannabinoids appeared to have a better correlation with urine as compared to other drug classes, while Barbiturates had the highest specificity for oral fluids.

Table 1

Drug Categories	Urine NH–ForenTox Cutoff ng/mL	Oral NH-Foren Tox Cutoff ng/mL	No. Tests	Urine "+"	OF "+"	DRE Opinions	Subject Admission
Benzodiaz	100	1.0	11	11	2	3	2
COC	100	5.0	16	13	14	4	4
THC	20	1.0	17	16	12	8	10
Opiates	200	10	8	8	4	8	5
Methamph	300	40	4	4	1	1	2
Barbiturate	200	20	3	1	3	2	2
Methadone	300	5.0	3	3	1	3	3
PCP	25	1.0	ND	-	-	-	-
Amphet	300	100	ND	-	-	-	-

Key Words: DRE, Oral Fluids, Urine Drug Screen, EIA

DRUG DETECTION IN HAIR: ASSESSMENT OF LEVELS FROM LARGE VOLUME SCREENING AND CONFIRMATION TESTING

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In recent years understanding of the benefits of the use of hair in drug testing has increased, particularly its advantages over urine, and more recently, oral fluid. The key benefit is the long time window of detection. This understanding together with reliable techniques has contributed to the dramatic increase in the use of hair samples in the detection of drug use. Amongst the wide variety of sectors that currently use hair for the detection of drugs, the largest are clinics, family law firms, the police and various organisations for workplace testing. The main purpose is to verify drug use or monitor drug abstinence. Very often when the analysis is performed and the results issued, the most common question that is raised is: what do the levels detected tell me about how much drugs were used by the person being tested? Because it is difficult to establish an accurate estimation of the amount of dose taken in relation to levels of drug in hair, the levels of drugs detected in hair are best used as a guide to changes in use in the individual when sectional analysis is performed or two different periods are compared in the same individual.

Nonetheless, it can be very useful, as a guide when writing reports or communicating with clients, to compare results obtained from a hair test from an individual's hair sample with results obtained in other samples from a large group of people. Hair samples (N=12,218) from various sources were received by Tricho-Tech for screening for drugs over a two-year period and analysed using the same method. All samples were washed, extracted then screened using coated-plate ELISA test for each drug group (Immunalysis Corporation, Pomona, CA, USA) and processed by an automated analyser Triturus (Grifols, Cambridge, UK) at a cut-off of 0.5 ng/mg (Table 1). Samples above the cut-off were submitted for confirmation analysis by Gas Chromatography-Mass Spectrometry (GC-MS or GC-MS/CI) using HP5973 (Agilent, Berkshire, UK) or Varian Inc. Saturn for GC-MS/MS (Walton-on-Thames, UK). The dynamic range was from 2 to 160 ng/mL. Three ions for the drugs and two ions for the internal standards were monitored.

Table 1. Hair samples analysed by ELISA and GCMS with confirmation rates for each drug group.

	Screened	GCMS		Confirmation Rate	
Group	N	N	%	N	%
Amphetamines	7355	4128	(56%)	1839	(45%)
Benzodiazepines	5804	1879	(32%)	1415	(75%)
Buprenorphine	786	113	(14%)	63	(56%)
Cocaine	8919	3150	(35%)	2490	(79%)
Methadone	6127	1893	(31%)	1666	(88%)
Opiates	8970	4164	(46%)	2901	(70%)

Percentiles 25%, 50% (Median), 75%, 95% and maximum levels for all analytes in each drug group were calculated and will be presented. Results for Cocaine Group are shown in Table 2.

Table 2. Results showing Percentiles and maximum levels in ng/mg of hair for Cocaine group

Analyte	25%	50%	75%	95%	Maximum	N
AEME	0.3	0.8	1.6	5.5	34.8	305
Benzoylecgonine	0.4	1.1	3.6	16.5	163.7	1988
Cocaethylene	0.3	0.5	1.2	4.1	9.4	339
Cocaine	0.9	2.9	11.9	59.5	814.2	2483

Using Cocaine as an example of drug levels by sector, median levels were: Clinical=6.9 ng/mg; Police=5.0 ng/mg; Medico-Legal=2.5 ng/mg and Employment =0.4 ng/mg. A consistent pattern was seen for the other drug groups.

The results show a wide range of levels for all analytes, frequency and distribution. Thus, this assessment of levels provides an important comparative aid for interpreting results of detected drugs in samples of hair.

Keywords: Drugs, Hair, GC-MS/MS

EVALUATION OF A RAPID ORAL FLUID POINT OF CARE TEST FOR MDMA

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Recreational use of 3,4-methylenedioxymethamphetamine (MDMA) either alone or in combination with other drugs such as alcohol and cannabis has become increasingly popular among young people throughout the UK, Europe and the US. The Cozart® RapiScan oral fluid drug test system has been use successfully for monitoring compliance within treatment centres, criminal justice settings and more recently at the roadside.

The aim of this study was to evaluate the Cozart® RapiScan test cartridge developed for the detection of MDMA and methamphetamine in oral fluid. Fifty oral fluid samples were obtained from drug addicts attending drug dependency units and were screened initially using the Cozart® microplate ELISA for amphetamines. All samples were then screened using the Cozart® RapiScan test cartridge for MDMA followed by confirmation by gas chromatography-mass spectrometry (GC-MS).

A total of 23 samples screened positive and were confirmed for MDMA either alone or in combination with amphetamine. A further 15 samples were confirmed negative for all amphetamines by GC-MS and 14 of these samples also screened negative using the Cozart® RapiScan. The false positive sample was investigated further and was found to contain an extremely high concentration of pseudoephedrine. The remaining 12 samples were confirmed positive for amphetamine only. Six samples screened negative and the other six screened positive due to the extremely high concentrations of amphetamine in these samples. No samples tested in this study contained methamphetamine.

The Cozart® RapiScan Drug Test System for the detection of MDMA correctly identified 100% of MDMA positive samples ranging from 53ng/mL to concentrations in excess of 180ng/mL. False positive results were only observed when samples contained extremely high concentrations of amphetamine or pseudoephedrine, which is consistent with the cross reactivity profile of the drug test.

Keywords: MDMA, Oral fluid, Onsite Test

ALCOHOL TESTING BY AN ONSITE ONE-STEP ORAL FLUID ALCOHOL AND DRUG COMBINATION TEST DEVICE – ORATECTPLUS™

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Aims: The objective of this study is to compare the alcohol detection of OratectPlus™, an onsite oral fluid alcohol and drug combination test device with Alco-screen™ – a saliva alcohol test.

Methods and Results: Oratect™ is a one-step rapid drug screen that integrates oral fluid collection, drug testing and confirmation sampling in one single device. OratectPlus™ is a new generation of Oratect that includes alcohol testing with drug testing and provides faster collection and test times. The new device consists a cap and a plastic housing containing a collection pad that is connected to an alcohol test pad on one side and two lateral flow immunoassay test strips that test for 6 abused drugs on another side. To run the test, the collection pad end of the device is inserted into the donor's mouth for one to two minute till red color fluid starts to appear on both lateral flow test strips. The color on the alcohol test pad is noted immediately. Negative alcohol level does not change the original tan color pad. Presence of bluish gray color on this pad indicates alcohol level at or exceeding 0.02% blood alcohol concentration. Drug test results are available from the two lateral flow strips after 3 minutes with the absence of red lines indicating positive results. In a field trial, forty individuals without drinking any alcohol were all tested negative with the OratectPlus and Alco-screen. Another forty individuals allowed to consume beer and then tested for alcohol within 20 minutes were all found to test positive for both OratectPlus and Alco-screen. Non-alcoholic drinks including sodas, tea and coffee were found not to interfere with the test result.

Conclusion: OratectPlus is shown to be a viable onsite device for testing alcohol use.

Keywords: Alcohol Testing, Oral Fluid, Drugs of Abuse

Δ9-TETRAHYDROCANNABINOL (THC) IN SWEAT PRIOR TO AND FOLLOWING CONTROLLED ORAL ADMINISTRATION OF HEMP OIL AND MARINOL™ TO CANNABIS USERS

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Six healthy individuals with a history of cannabis use resided on the secure clinical research unit of the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health for the 10 to 12 week study. Participants provided informed consent for this Institutional Review Board-approved oral cannabinoid administration protocol. This was a randomized, double blind, double dummy, placebo-controlled within–subject study. Sweat was collected throughout the study with PharmCheck™ sweat patches worn for 24 hours or 7 days. The initial washout phase of this study lasted from admission until subjects' urine cannabinoid concentrations were below 10 ng/mL by fluorescence polarization immunoassay. Sweat patches worn during the initial washout phase enabled investigation of excretion of THC resulting from previously self- administered cannabis. The dosing phase of the study followed the initial washout phase, during which the participants were dosed three times a day for five consecutive days followed by a ten-day washout period before the next dosing session. All subjects ingested commercially available hemp seed oil of differing THC concentrations: 0, 0.39, 0.47 (contained within capsules), and 14.8 mg THC/day. In addition, 7.5 mg/day dronabinol or Marinol™, was administered as a positive control. PharmChek™ sweat patches collected throughout the study were frozen at –20°C until analysis. Patches were extracted in methanol: 0.2M sodium acetate buffer, pH 5.0, 3:1 v/v, followed by solid phase extraction using CleanScreen, ZSTHC020, (United Chemical Technologies, Bristol, PA). Dried extracts were derivatized with trifluoroacetic acid (TFAA) and analyzed using gas chromatography negative chemical ionization mass spectrometry (GC–NCI–MS) for THC. Daily and weekly sweat patches collected during the initial washout period were analyzed along with weekly patches collected during and following dosing. None of the patches worn during or following dosing tested positive for THC with a cutoff of 0.4 ng/patch. Patches worn during the first week of residence on the research unit, reflecting drug excretion from previously self-administered cannabis tested positive for THC in two individuals. The concentrations of THC were 0.51 and 0.82 ng THC/patch for patches worn for the first seven days on the research unit. These participants self-reported cannabis smoking an average of 3.0 and 4.5 days per week and claimed to have last used cannabis one to two days prior to study enrollment. However, these usage histories did not differ significantly from those reported by the other four subjects and whose sweat patches tested negative for THC. In conclusion, this study demonstrates that oral administration of THC at, as high a dose as 14.8 mg/day for 5 consecutive days did not produce positive sweat tests for cannabinoids with a 0.4 ng THC/patch cutoff in patches worn for seven days. The cannabinoid confirmation cutoff for THC in sweat as proposed by the Substance Abuse Mental Health Services Administration is 1 ng THC/patch. It is important to note that the bioavailability of oral THC has been estimated to be 6 to 20% and plasma THC concentrations did not exceed 6.5 ng/mL following any of the dosing regimens during this study. These data indicate that previously administered THC in daily cannabis smokers did not produce positive cannabinoid sweat tests when patches were applied at the beginning of abstinence and worn for 7 days if a 1.0 ng THC/patch cutoff is used. In addition, no sweat patches were positive at this cutoff when up to 14.8 mg THC/day for 5 consecutive days was ingested by cannabis users. Ingestion of commercially available hemp oil products and Marinol up to 7.5 mg/day did not produce positive sweat cannabinoid tests.

Keywords: Δ9-Tetrahydrocannabinol, Oral Cannabinoids, Sweat Patch

QUANTITATIVE ANALYSIS OF DEXTROMETHORPHAN, CARISOPRODOL AND THEIR METABOLITES IN HAIR BY GC-MS

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Dextromethorphan, which is used as an antitussive agent, and carisoprodol, which is used as a muscle relaxant, have been widely abused among young people in Korea. Even though these are known to be very safe drugs for therapeutic use, people abused them for their hallucinogenic properties. Due to the serious abuse liability potential of these drugs, our government recently decided to control them as psychotropic agents. It was necessary for us to establish a detection method for dextromethorphan and carisoprodol and their metabolites in hair to demonstrate the abuse of these drugs. A method was established to simultaneous quantify dextromethorphan, dextrorphan, 3-methoxymorphinan, carisoprodol and meprobamate in hair samples. Analytes were extracted from fine cuttings of hair in 1% HCl in methanol for 16 hours. After evaporation under N_2, residues were separated on a HP - 5MS column using a 16 min program and identified by mass spectrometry in the SIM mode (EI-GC-MS).This method was validated for the recovery, linearity of calibration, within-and between-day precision, accuracy, limit of detection and quantification. Calibration curves exhibited correlation coefficients > 0.99. Within and between-run precision was calculated at 8, 80 and 400 ng/mg of drug or metabolite in hair with coefficients of variation less than 14 %, except for meprobamate (17%). Accuracy at the same concentrations was 14% of target for all analytes except meprobamate (18%). Recoveries at 10 and 100 ng of drug or metabolite in hair were 88 to 117 %. With this method, we performed quantitative analysis of dextromethorphan and carisoprodol in abuser's hair. The concentrations of dextromethorphan, dextrorphan and 3-methoxymorphinan ranged from 1.4 ~ 225.0, 1.3 ~ 44.7, and 0 ~ 42.7 ng/mg in hair from 31 donors studied, respectively. The level of carisoprodol and meprobamate were 2.3 ~ 52.9 and 11.3 ~ 365.1 ng/mg respectively in 11 hair samples. We present a validated, sensitive and specific GC-MS method to simultaneously quantify dextromethorphan, carisoprodol and metabolites in hair.

Keywords: Dextromethorphan, Carisoprodol, Hair Analysis

EVALUATION OF THE IDS ONE-STEP ELISA KIT FOR THE SCREENING OF KETAMINE IN HAIR

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An evaluation study was conducted with the commercially available ketamine microtiter plate enzyme-linked immunoassay (ELISA) of International Diagnostic System (IDS), USA, in the screening of ketamine in hair. The assay principle is based on the competitive binding between the ketamine-enzyme conjugate and the free ketamine extracted from the hair for a limited number of antibody-binding sites. Aliquots of 20 µl of the sample or calibrator was added to the antibody-coated microwell followed by 100 µl of diluted enzyme conjugate. After an incubation period of 30 min at room temperature, any unbound material was removed with washing. An enzyme substrate was then added and further incubated for 15 min for the colour development. An acid stop solution was added to stop the reaction. The absorbance is measured at 450 nm with 650 nm as the reference filter. The absorbance obtained is inversely proportional to the amount of ketamine present in the sample.

During the sample preparation, the hair specimens were washed and decontaminated before being pulverized in a Retch ball mill. About 25 mg of the pulverized hair was digested overnight at 45°C in 1 ml of 0.5 M hydrochloric acid. The samples were allowed to cool before the addition of 1 ml of 0.5 M sodium hydroxide and 1 ml of phosphate buffer solution (pH8). 20 µl of the clear supernatant were used for the immunoassay.

The objective of this study is to determine if a commercially available ELISA system was sufficiently sensitive for the routine screening of ketamine in hair. We evaluate the immunoassay performance in terms of precision, accuracy, efficiency, sensitivity and specificity. The limit of detection (LOD) of the kit was found to be 0.6 ng/mg. The linearity range for ketamine was found to be up to 4.3 ng/mg using spiked hair samples. The intra-day % CV (within-day precision) for ketamine at concentrations of 0.6, 0.8 and 1 ng/mg was found to be from 3.73 % to 6.12 %. Interference study was evaluated by measuring the extent of cross reactivity of various common drugs of abuse such as opiates, 11-nor-Δ^9-THC-9-carboxylic acid, amphetamines and their ring analogues, cocaine and its metabolites, benzodiazepines, buprenorphine and its metabolites, LSD and its metabolites with ketamine. All the drugs above do not interfere with the detection of ketamine using ELISA. Only a low cross-reactivity was observed for low concentrations of norketamine.

A total of 62 hair segments from suspected ketamine abusers were screened using the ELISA test kit. These hair specimens were obtained from suspected ketamine abusers. The effciency, sensitivity and specificity of the immunoassay were evaluated in comparison with gas chromatography/ mass spectrometry (GC/MS) using these hair specimens. Confirmatory cut-off for ketamine was 0.6 ng/mg. True positives, true negatives, false positives, and false negatives were determined using immunoassay cut-off at 0.8 ng/mg and 1.0 ng/mg. An optimum immunoassay cut-off concentration of 1.0 ng/mg was determined for the kit. At this cut-off, a total of 55 hair samples were screened positive and 7 screened negative. Comparing with GC/MS results, 2 false negatives and no false positives were determined. The immunoassay exhibited an efficiency of 96.7 %, a sensitivity of 96.4 % and a specificity of 100 %.

The results collected support that the IDS One-Step™ ELISA test kit provides a suitable preliminary screening procedure for the detection of ketamine in hair specimens. It offers a rapid, sensitive, and effective method to determine the presence of ketamine in hair in suspected ketamine abusers.

Keywords: ELISA, Ketamine, Hair Analysis

GAS CHROMATOGRAPHY-HIGH-RESOLUTION MASS SPECTROMETRIC METHOD FOR DETERMINING METHAMPHETAMINE AND ITS MAJOR METABOLITE AMPHETAMINE IN HUMAN HAIR

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Objective: The purpose of this study is 1) to effectively eliminate the biological background or interference by cosmetic treatment and 2) to develop a suitable confirmatory procedure for analyzing methamphetamine (MA) and amphetamine (AMP) in human hair using a gas chromatography high-resolution mass spectrometric (GC-HRMS) technique.

Nature of the study: GC-HRMS method development for detecting MA and its metabolite AMP containing an extremely low concentration in human hair, and eliminating co-eluted compounds in cosmetically treated hair samples.

Material and methods: We measured the total length of hair samples, and noted special features such as coloring, bleaching, etc. Before we analyzed hair samples, we 1) washed them twice with 10 mL water and 10 mL acetone, 2) then dried them under a fume hood, 3) finely cut the hair with scissors into small fragments below 1 mm, and 4) weighed each hair sample. We then transferred thirty milligrams of hair to a Teflon-faced, rubber-lined screw-cap test tube (16 × 100 mm). To extract analytes from the specimens, we added 2 mL of methanol-5 M hydrochloric acid (20:1, *v/v*) containing 100 ng of AMP-d_5 and MA-d_5 as internal standards into test tubes. Then, we directly extracted the hair samples under ultrasonication for 1h and left them to stand at room temperature overnight. The hair was filtered off and filtrate was evaporated under a nitrogen stream in a TurboVap LV evaporator (Zymark Corp., USA). We dissolved the dried sample in 50 μL of trifluoroacetic anhydride (TFAA) and 50 μL of ethylacetate and placed it in a dry heating block for the derivatization at 70°C for 30 min. The mixture was allowed to cool to room temperature. The residue was reconstituted with 40 μL volume of ethylacetate. We injected an aliquot (1 μL) of the sample solution into GC-MS.

Results: With the HRMS method the limits of detection (LOD) were 9 pg/mg for MA and 21 pg/mg for AMP using a 30 mg hair sample, and the SIM responses were linear with coefficients of correlation ranging from 0.9998 to 0.9999. The recoveries were found to be 91.1-92.3%. By using HRMS (resolution of 5000), we improved the detection sensitivity due to eliminating the biological background. The LODs for MA and AMP were 2.4-4.4 times lower than low-resolution mass spectrometry (LRMS). We applied the described method to hair samples from suspected MA abusers. In nineteen of the thirty hair samples not detected by the LRMS SIM technique, we confirmed AMP using an accurate HRMS SIM measurement of diagnostic ions. In cases where there is a low concentration of AMP in the hair, high resolution SIM is useful to eliminate chemical background, which is present in single-quadrupole LRMS due to solvent and/or matrix ionization. The HRMS technique makes the detection of rather low concentrations of drugs in hair samples possible. In this experiment, the hair samples analyzed using GC-HRMS are not specially prepared and are the same as those analyzed by GC-LRMS.

Conclusion: The proposed GC-HRMS method shows a high sensitivity and specificity. This method can also be used as a suitable analytical tool for identifying AMP as major metabolite of MA in human hair, where identifying metabolites at a low amount in hair samples is requested, and especially in cosmetically treated hair.

Keywords: Hair, GC-HRMS, Methamphetamine, Amphetamine

A MIXED MODE SOLID-PHASE EXTRACTION METHOD FOR THE LC/MS DETERMINATION OF DRUGS OF ABUSE IN PRESERVED SALIVA

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Saliva is a less invasive alternative to urine or blood in forensics analysis for drugs of abuse. Because drug residues are typically less concentrated in saliva than in other biological fluids such as blood or urine, quantification limits for saliva samples must be considerably lower than for urine, in the low ng/mL range. Compared with GC/MS, LC/MS is a simpler analytical approach requiring no derivatization. However, the saliva collection fluid contains ingredients (stabilizers and preservatives) that present some interference problems for LC/MS. A mixed-mode (cation-exchange) SPE cartridge, Oasis MCX, provided effective cleanup suitable for LC/MS determination of basic drugs of abuse in preserved saliva. The drugs studied were cocaine, codeine, morphine, amphetamine, methamphetamine, ecstasy (MDMA) and phencyclidine (PCP). MDMA-d5 was used as an internal standard. Calibration was accomplished in the range from 5 to 250 ng/mL. Correlation coefficients (r^2) for all constituents were better than 0.998. Reproducibility for all constituents was better than 10 % (RSD) for 6 replicate samples fortified at the 10 ng/mL and 50 ng/mL levels. Recovery was better than 85 % for all constituents.

Keywords: Saliva, LC/MS, Drugs of Abuse

COCAETHYLENE: A POTENTIALLY LETHAL TOXICANT

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Introduction: Cocaethylene (CE) is an active metabolite of cocaine and ethanol, produced by the liver in a majority of cocaine abusers who concomitantly abuse ethanol. Though the mechanism of death associated with cocaine overdose has historically been relative to vasospasm and cardiac/cerebral infarct and failure, we propose a role for CE as a toxicant to the vascular endothelium. We investigated the effects of CE on microvascular endothelium to further characterize mechanisms of vasculotoxicity and associated tissue ischemia.

Methods: An *in vitro* model of microvascular endothelial CE exposure (1mM) was used. Cytotoxicity assays included viability and LDH release analysis. Morphological (silver stain) and electrical/resistance analysis was used to determine the effect of CE on monolayer permeability. Analysis of variance for $n \geq 3$ was significant when $p<0.05$.

Results: Exposure of CE to microvascular endothelium, though not lethal, resulted in cellular injury that increased monolayer permeability. Viability of CE-treated monolayers (93.1 ± 1.9%) was not significantly different than controls (90.4 ± 3.8%). LDH release assay produced no significant results, with the average LDH concentrations measuring 173 ± 33 U/L (control) and 157 ± 43 U/L (CE-treated), respectively. Monolayers treated with CE for 1 hour (and up to 72 hours) showed large intracellular gap formations, whereas controls at each time point maintained intact intercellular borders. Resistance (inversely proportional to permeability) decreased in CE-treated monolayers (1090.25 ± 162.5 Ohm, versus 1319.48 ± 59.7 Ohm for controls) within 4 hours. This effect was maintained for the 72 hours of analysis.

Conclusions & Significance: We conclude that CE is capable of direct interaction with the vascular endothelium in a manner that promotes vascular disruption and tissue ischemia via increased permeability and edema. Such activity increases the likelihood for ischemia of high-demand tissues fed primarily by small blood vessels (brain, kidneys, intestines), and vasculitis, and could result in a life threatening systemic ischemic crisis when coupled with the other toxic effects of cocaine/CE (vasoconstriction, altered neurotransmission and neurotransmitter signaling). These results also show that CE could be more important to death investigation than previously thought.

Keywords: Cocaethylene, Cytotoxicity, Microvascular Endothelium

FORENSIC ENTOMOTOXICOLOGY: A STUDY IN THE DEPOSITION OF BARBITURATES INTO THE LARVAE OF THE BLACK BLOW FLY, *PHORMIA REGINA*

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Due to events in severe decomposition, either no soft tissue remains on which to perform a toxicological analysis or putrefactive fluids complicate and interfere with the analysis of the soft tissues. The purpose of this experiment was to study the trends in the deposition of barbiturates into the larvae of the black blow fly, *Phormia regina*, in order to better understand the value of entomological evidence as toxicological specimens. Drug deposition was analyzed by linear regression to find a correlation between whole larvae drug concentration and food source drug concentration. *P. regina* larvae were raised at 21°C on pork homogenized with three concentrations of barbital (200, 400, 800mg/kg), phenobarbital (20, 40, 80mg/kg), pentobarbital (20, 40, 80mg/kg), and thiopental (15.5, 31, 62mg/kg). The middle dosage of each drug reflected the LD_{50} of the drug in rabbits. At the end of the feeding stage, the larvae were harvested, washed, and frozen. Ten larvae were subsequently homogenized, diluted in 2 ml water, and subjected to a liquid-liquid extraction. The extracted drugs were derivatized with MethElute and analyzed by GC/MS. In the concentration ranges investigated, the concentrations of barbital, phenobarbital, pentobarbital, and thiopental found in the larvae strongly correlated with the concentration of the drug in the food source (R^2 = 0.9976, 0.9914, 0.8938, and 0.9095, respectively). Larvae feeding on pork homogenized with 800mg/kg of barbital accumulated 1.8 times more drug than those feeding on 200mg/kg. Larvae which fed on the high concentrations of phenobarbital, pentobarbital, and thiopental accumulated, respectively, 3.1, 5.4, and 68.5 times more drug than those that fed on the low concentrations of drugs. This data indicated that as the lipophilicity of the drug increased, the larvae accumulated significantly more drug.

Keywords: Entomology, Toxicology, Barbiturates

DETECTION OF COTININE IN EXHALED BREATH BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY: A PRELIMINARY STUDY

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Exhaled breath analysis is a very useful diagnostic tool in the fields of medicine and forensic science. Exhaled breath is routinely used to detect markers of disease, as well as correlate ethanol concentrations with effects on human performance and behavior (DUI). Compared to other biological specimens, the collection of exhaled breath is simple and non-invasive, with minimal exposure to infectious material. Because exhaled breath is a physiological waste product, the quantity available exceeds those of other biological matrices such as blood and urine. In addition, little sample preparation is required. Finally, exhaled breath accounts for all routes of drug exposure including oral ingestion, injection, inhalation, and dermal absorption.

This is a preliminary study to determine if cotinine, an oxidative metabolite of nicotine, is detectable in exhaled breath of humans. Cotinine was chosen as a metabolic marker of nicotine use because of its long half-life compared to nicotine. Exhaled breath specimens were collected onto commercially-available XAD-4 traps (Supelco) and analyzed by gas chromatography-mass spectrometry (GC-MS). The resin, XAD-4, is a polymeric adsorbent available as beads within a small glass tube (70 mm x 6 mm). Upon collection of the exhaled breath, the resin was removed, fortified with d_3-cotinine (Cerilliant Corporation), and sonicated in methanol for 30 minutes. The organic layer was separated from the resin and dried under a gentle stream of nitrogen at 40°C. The residue was reconstituted in phosphate buffer (pH 6). The cotinine was isolated from the buffer utilizing CleanScreen® solid-phase extraction (SPE) cartridges (ZSDAU020) manufactured by United Chemical Technologies, Inc. Following elution from the SPE cartridges using a solution of methylene chloride, isopropanol, and ammonium hydroxide, the extracts were dried under a gentle stream of nitrogen at 40°C and reconstituted in methanol. The extracts were subjected to GC-MS analysis using an Agilent Technologies 6890N series gas chromatograph equipped with a Agilent Technologies 5973 network mass spectrometer operated in electron ionization and selected ion monitoring (SIM) modes. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Automated injections (2-3 µL) were made onto a capillary GC column. The ions (*m/z*) monitored for cotinine were 98.1, 175.1 and 176.1, and the ions (*m/z*) monitored for d_3-cotinine were 101.1 and 179.1.

Initial studies have demonstrated cotinine in the exhaled breath of cigarette smokers, while cotinine was not detected in control (non-smokers) exhaled breath. These data will serve as a model for detection of other compounds (drugs and their metabolites) present in exhaled breath which may be beneficial for many other medical, forensic, and toxicological applications.

Keywords: Cotinine, Exhaled Breath, GC-MS

"THE MECONIUM PROJECT": AN ITALIAN-SPANISH JOINT STUDY TO ASSESS EXPOSURE TO ILLICIT DRUGS DURING PREGNANCY AND BIRTH OUTCOMES

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The accurate assessment of fetal exposure to drugs of abuse through the objective measure of biomarkers could provide the basis for appropriate treatment and follow-up of new-borns, which can present symptoms of drug withdrawal. Furthermore, information regarding the real prevalence of drugs of abuse use during pregnancy could also be disclosed.

For the first time in Europe the "Meconium Project" aimed to estimate the prevalence of drug use by pregnant women and the effects of exposure to illicit drugs during pregnancy on the mother, fetus, and infant.

Between September 2002 and February 2004, among the mother-infant dyads from the Hospital del Mar, the fourth biggest hospital of the city, 1439 dyads met eligibility criteria and 1151 (79%) of those eligible agreed to participate in the study. Exposure was defined as an admission of use of cocaine, opiates or amphetamines by structured interview or the presence of drugs and their metabolites in 24 and 48 h meconium as determined by use of liquid chromatography-mass spectroscopy assay. Nonexposure was defined as a negative drug use history by interview and a negative analytical result. When exposure could not be confirmed, such as when meconium was not obtained or was inadequate for confirmatory analysis, the mother-infant dyad was excluded (n =288).

Preliminary results on the analyzed 497 mother-infant dyads showed that structured interview disclosed a 0.8, 1.8 and 2.2% mothers exposed to opiates, cocaine and both drugs while only one mother declared ecstasy consumption. Meconium analysis showed that prevalence of opiates, cocaine and combined drugs exposure was 9.1, 3.8 and 10.7%, respectively and confirmed the case of ecstasy use. Arecoline, the main Areca nut alkaloid, was found in meconium specimens from 4 out of 10 Asiatic newborns, whose mothers declared areca nut consumption during pregnancy.

A similar percentage of drug-exposed mothers were found between Spanish, American and African women (11.5, 9.9 and 8.6%, respectively), while only a 6.3% Asiatic women resulted drug consumers. Exposure status caused a significant low birth weight in newborns from mothers exposed to cocaine (mean and S.D.= 2848 $\pm$ 315 g in newborns from cocaine mothers vs 3262 $\pm$ 504 g in newborns from non-exposed mothers). Other somatometric differences included crown-heel height.

Of the 4 newborns exposed to arecoline, one showed a low birth weight, low intrauterine growth, hyporeflexia, hypotonia and a second presented a withdrawal neonatal syndrome by Finnegan test.

In conclusion, these preliminary findings indicate a prevalence of opiates, cocaine and amphetamines consumption during pregnancy in this mixed mediterranean and non-european population lower that that reported in the North-american pregnant women. The higher sensitivity of meconium analysis, already demonstrated, in comparison to maternal interview, was confirmed making this test ideal for screening maternal and fetal exposure to conventional and non-conventional drugs of abuse during pregnancy.

Keywords: Drugs of Abuse, Exposure, Pregnancy

METHAMPHETAMINE AND AMPHETAMINE CONCENTRATIONS IN MECONIUM OF NEONATES OF WOMEN ENROLLED IN THE IDEAL STUDY OF IN UTERO METHAMPHETAMINE EXPOSURE

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Background: Tobacco, cannabis and methamphetamine (MA) use by pregnant women can negatively impact child development. The Infant Development, Environment, and Lifestyle (IDEAL) study is a multi-center, longitudinal investigation of the effects of prenatal methamphetamine exposure. Meconium, a useful matrix for identifying in utero drug exposure, was employed to identify gestational drug use. Little published data are available on MA detection in meconium.

Objective: To characterize the detection and quantification of MA, tobacco and cannabis in meconium compared to self-report.

Method: Mothers who used tobacco, cannabis or alcohol during pregnancy were included in both MA-exposed and control groups. Meconium samples were analyzed by EMIT (USDTL) for cannabinoids, cocaine, opiates, amphetamines with cutoff concentrations of 40, 75, 150 and 500 ng/g, respectively. Positive samples were confirmed by GC/MS with cutoffs of 2 ng/g for 11-nor-Δ9-carboxy-THC and 5 ng/g for cocaine, benzoylecgonine (BE), m-OH-BE, and cocaethylene, morphine, codeine, hydrocodone, hydromorphone, and MA, amphetamine (AMP), and MDMA. Tobacco use was identified with an ELISA cotinine screen (International Diagnostics Systems Corp., cutoff 10 ng/g).

Results: Of the 13,808 mothers screened, 1631 were consented and 176 enrolled. MA exposed mothers (n=84) were identified by self-report of gestational MA use and/or GC/MS confirmation of MA, AMP, and/or MDMA in infant meconium. Comparison participants (n=92) were matched by race, birth weight, maternal education and type of insurance, denied amphetamines use and had negative meconium results. Among the 1631 mothers, self-reported use rates were 5.2% (amphetamines), 25% (tobacco) and 5.9% (cannabis). Positive meconium screening rates were 3.6% for any amphetamine, 20% cotinine and 11.2% cannabis. For specimens that screened positive, 40.7% of amphetamines and 20.2% of cannabis specimens were confirmed. EMIT sensitivity and specificity were checked by reanalyzing 35 specimens from babies of women who reported MA use but had a negative screen and 35 specimens from the control group that also tested negative. All controls' meconium specimens were negative by GC/MS (5-ng/g), while 4 of 35 specimens from self-reporters that screened negative were found positive by GC/MS, albeit at low concentrations (MA 8 – 52 and AMP 5 – 26 ng/g meconium).
Users reported administering MA by the following routes: 48% smoked; 9% injected, 11% sniffed, 6% ingested and 26% used multiple routes of administration. On average, 68% of the meconium from neonates whose mothers reported 3rd trimester use had detectable MA, while detection rates were ≤10% for self-reported use during the 1st and/or 2nd trimesters. Mean±SD, median and range of MA concentrations were 3674 ± 3406, 2623, 479 to 13,431 ng/g meconium and AMP 569 ± 543, 403, 30 to 2000 ng/g meconium in infants whose mothers reported 3rd trimester use. However, the highest MA (19,376 and 16,976 ng/g) and AMP (2765 ng/g) concentrations were found in offspring born to women who reported MA use only in the 1st or 1st and 2nd trimesters, raising questions about the self-report. The log transformed meconium MA concentrations significantly correlated with the frequency of MA use in the 3rd trimester (r=0.645, P=0.004), although variability prevents prediction of frequency of use for an individual mother. AMP was always detected in MA positive meconium. In 55% of the GCMS positive samples, the ratios of amphetamine to MA were 0.1 to 0.2; 14% were less than 0.1 and 18% were 0.2 to 0.3.

Limitations: Data are preliminary as the IDEAL study continues to recruit; larger samples in the future may strengthen or refute interim observations.

Conclusions: Meconium analysis for MA is a useful adjunct to self-report for identification of MA exposure; however, the greatest sensitivity was achieved with specimens collected from offspring of women who reported use in the 3rd trimester. Further research is needed to determine if there are additional MA metabolites in meconium that could improve the identification of MA-exposed infants.

Keywords: Methamphetamine, Meconium, In Utero Exposure

PASSIVE CANNABIS SMOKE EXPOSURE AND ORAL FLUID TESTING

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Illicit cannabis use continues to be highly prevalent in the United States. Oral fluid testing for Δ9-tetrahydrocannabinol (THC) provides a convenient means of detection of recent usage. In this study, the risk of positive oral fluid tests from passive cannabis smoke exposure was investigated by housing four cannabis-free volunteers in a small, unventilated and sealed room with an approximate volume of 36 m^3. Five active cannabis smokers also were present in the room and each smoked a single cannabis cigarette which contained an average of 1.75% THC. Cannabis smoking occurred over the first 20 minutes of the study session. All subjects remained in the room for approximately 4 hrs and provided oral fluid and urine specimens at designated times. Oral fluid specimens were collected with the Intercept DOA Oral Specimen Collection Device before the start of the session and periodically throughout the session. Three urine specimens were collected (0, 20 and 245 min). In addition, three air samples were collected (first 5 min during smoking, for 20 min immediately following smoking, and at 65-100 min after start of session) for measurement of THC concentrations in air. All oral fluid specimens were screened by enzyme immunoassay (EIA) for cannabinoids (cutoff concentration = 3 ng/mL) and tested by GC-MS-MS for THC (LOQ/LOD = 0.25 ng/mL). All urine specimens were screened by EIA for cannabinoids (cutoff concentration = 50 ng/mL) and tested by GC-MS-MS for THCCOOH (LOQ/LOD = 1 ng/mL). Air samples were measured for THC by GC-MS (LOD = 1 ng/L). A total of eight oral fluid specimens (collected 20 to 50 min following initiation of smoking) from the four passive subjects screened and confirmed positive for THC at concentrations ranging from 3.6-26.4 ng/mL. Two additional specimens from one passive subject, collected at 50 and 65 min, screened negative but contained THC in concentrations of 4.2 and 1.1 ng/mL, respectively. All subsequent specimens for passive participants tested negative by EIA and GC-MS-MS for the remainder of the 4 hr session. All urine specimens collected during the session from the passive participants screened and confirmed negative at LOQ/LOD by GC-MS-MS with the exception of one subject whose specimen collected at the end of the session contained 3.4 ng/mL of THCCOOH by GC-MS-MS. In contrast, oral fluid specimens collected from the five cannabis smokers generally screened and confirmed positive for THC throughout the session at concentrations substantially higher than observed for passive subjects. Urine specimens from cannabis smokers also screened and confirmed positive at conventional cutoff concentrations. Based on the limited air sample measurements, it was estimated that passive subjects inhaled a total of approximately 0.05 mg of THC during the exposure session. A biphasic pattern of decline for THC was observed in oral fluid specimens collected from cannabis smokers, whereas a linear decline was seen for passive subjects suggesting that initial oral fluid contamination is cleared rapidly and is followed by THC sequestration in the oral mucosa. It is concluded that the risk of positive oral fluid tests from passive cannabis smoke inhalation is limited to a period of approximately one hour following exposure.

Keywords: Cannabis, THC, Passive, Oral Fluid

DISPOSITION OF Δ9-TETRAHYDROCANNABINOL IN ORAL FLUID AND PLASMA AFTER CONTROLLED ADMINISTRATION OF SMOKED CANNABIS

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Understanding the relationship of Δ9-tetrahydrocannabinol (THC) concentrations in oral fluid and plasma is important in interpretation of oral fluid test results. Current evidence suggests that THC is deposited in the oral cavity during cannabis smoking. This "depot" represents the primary or sole source of THC found when oral fluid is collected and analyzed. In this research, oral fluid and plasma specimens were collected from six subjects following smoking of cannabis cigarettes containing 1.75% and 3.55% THC. There was at least one week between each cannabis administration. Plasma specimens were analyzed by GC-MS and paired oral fluid specimens were analyzed by radioimmunoassay (RIA). In addition, one individual's oral fluid specimens were also analyzed by GC/MS. These data are unique in that they represent simultaneous or near simultaneous collection of oral fluid and plasma specimens in subjects following controlled cannabis dosing. The first oral fluid specimen, collected from one subject at 0.2 hr following initiation of smoking, contained a THC concentration of 5800 ng/mL (GC-MS). By 0.33 hr, the THC concentration in oral fluid had fallen to 81 ng/mL. From approximately 0.3 hr through 4.0 hr, the mean (± SD) THC ratio of oral fluid to plasma THC concentrations was 1.18 (0.62) with a range of 0.5 to 2.2. Within 12 hr, both oral fluid and plasma THC concentrations generally declined below 1 ng/mL. RIA analyses of oral fluid specimens for six subjects demonstrated the same pattern of initial high levels of contamination immediately after smoking, followed by rapid clearing, and a slower decline over 12 hr. Mean THC oral fluid concentrations by RIA at 0.2 hr were 864 ng/mL and 4167 ng/mL compared to plasma concentrations of 52 ng/mL and 230 ng/mL at 0.27 hr following the low and high dose cannabis cigarettes, respectively. The similarity in oral fluid and plasma THC concentrations following the dissipation of the initial "contamination" indicates the likelihood of a physiological link between these specimens. Recent studies have shown that sublingual or transmucosal administration of pure THC results in direct absorption of intact THC into the bloodstream, thereby bypassing the gastrointestinal tract. The current study demonstrates that THC is deposited in the oral cavity and remains for up to 24 hr following cannabis smoking. The decline in THC oral fluid concentration over this time suggests that there may be absorption of THC into blood as previously shown with pure THC. Passive cannabis exposure studies appear to indicate that positive oral fluid tests for THC can occur shortly after cannabis smoke exposure, but results were negative within one hour. Consequently, when very recent passive exposure to cannabis smoke can be ruled out, it is concluded that a positive oral fluid test provides credible evidence of active cannabis use.

Keywords: Cannabis, THC, Oral Fluid, Depot

DETECTION OF DRUGS OF ABUSE USING ORAL FLUID WITHIN A PAROLE SETTING

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A study was conducted using rapid on-site and lab based oral fluid tests as well as urine for drugs of abuse detection in a probation facility that requires routine testing of participants. Individuals in this facility are required to routinely visit with a probation officer. As part of their visit a urine specimen is collected, any presence of drug is punishable and grounds for revocation of probation. This study was organized to compare new technologies for on-site and lab-based oral fluid tests comparing them to routine urinalysis. On-site oral fluids were determined using the UPlink Test System. UPlink is based on instrumented analysis of lateral flow chromatography using Up-Converting Phosphors. Lab-based oral fluids were determined using the Intercept System. The Intercept oral fluid collection device gathers an average of 0.4mL of oral fluids, which is express-sent to a laboratory. After isolation of the sample, Intercept specimens are screened using microplate assays followed by GCMSMS confirmation of positive samples. Urine analysis was performed using Microgenics Inc. reagents on a Hitachi 917. A total of 217 specimens were tested in oral fluids. A total of 207 specimens were tested with urine due to problems with subjects unable to provide a specimen. Positive samples were found in all fluids for all major classes of drugs including cocaine, THC, opiates, PCP and amphetamines. True Positives and True Negatives were judged based upon manufacturers recommendations for oral fluids and Substance Abuse and Mental Health Services Administration Federal workplace drug testing guidelines for urine, with the exception of opiates, which use a 300 ng/mL cutoff. Overall, the % agreement when comparing on-site UPlink and lab-based Intercept results to GCMSMS was 98.7% and 99.6% respectively. Overall % agreement between urine screening results and GCMS was 99.8%. These results demonstrated the analytical performance of either oral fluid test system was comparable to lab-based urine testing. However, collection of oral fluid negated the requirements for special facilities allowing probation officers to directly observe collections.

Keywords: Oral Fluid, Urine Drug Testing, Drugs of Abuse

DISPOSITION OF COCAINE AND METABOLITES IN HUMAN SWEAT FOLLOWING CONTROLLED COCAINE ADMINISTRATION

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The analysis of sweat for drugs of abuse offers a convenient, non-invasive technique for monitoring drug exposure. Sweat can be collected using the Pharmchek® Sweat Patch; an absorbent cellulose pad with adhesive backing that is worn on the body. The comparison of sweat test results to urinalysis indicates that sweat may be an acceptable matrix for drugs of abuse screening for drug treatment, criminal justice, and employment screening purposes.
Healthy volunteers (n=8), with a history of cocaine (COC) use, provided informed consent to participate in this IRB approved research study and resided on the closed ward throughout the 12-week study. After an initial three-week washout period, volunteers received 3 low dose (75 mg/70 kg) subcutaneous injections of cocaine HCl on every other day and, three weeks later, 3 high doses (150 mg/70 kg).

Pharmchek® Sweat Patches collected for up to 48 hours following the first low (n=82) and high (n=78) doses were isolated for cocaine and eleven metabolites by solid phase extraction and analyzed by GC/EI-MS. LOD and LOQ were 2.5 ng/patch for COC, ecgonine methyl ester (EME), benzoylecgonine (BE) and meta- and para-hydroxycocaine (m- p-OH-COC).

COC was the primary analyte detected in patches worn for 1-15 hours after the first low (26 of 82, 32% positive patches) and high (36 of 78, 46% positive patches) doses. COC Cmax ranged from 6-64 ng/patch and 5-375 ng/patch following low and high doses, respectively. Not only was there large variability in Cmax, but there also was wide variability for the time of Cmax. Several patches (2 of 27 for low dose and 6 of 26 for high dose) worn for four or less hours were positive for COC 24-48 hours after administration. Half of the participants had positive COC results for both low and high dose sweat patches applied 32 hours after dose and worn for 15 hours, demonstrating improved detection in patches worn for extended periods.

EME was detected in sweat patches collected from 3 participants after low dose (7% positive patches) and 4 participants after the high dose (13% positive patches) and was detected only in patches collected for the first 24 hours after dosing. Maximum EME concentrations ranged from 8-47 ng/patch and 5-51 ng/patch for the low and high dose, respectively. BE was detected in 6% of the low and 8% of the high dose patches with concentration ranges of 5-13 ng/patch after low dose and 6-12 ng/patch after high dose. One participant had a positive m-OH-COC patch (3 ng/patch) after high dose administration. In addition, m- and p-OH-COC were detected concurrently in the 2-4 hour (3 ng/patch m-OH-COC, 6 ng/patch p-OH-COC) and 8-23 hour (3 ng/patch m-OH-COC and 5 ng/patch p-OH-COC) patches collected from one participant after the high dose.

COC, BE and EME can be detected within 2 hours of drug administration but there is wide intra- and inter-subject variability in the amount detected and the time at which Cmax is reached. During the 24 hours after exposure COC can be detected in patches worn for short periods (1-2 hours) but after 24 hours the patch must be worn for longer periods to detect COC. EME was detected more often and at higher concentrations than BE; both were detected only within the first 24 hours after exposure.

Sweat testing for COC and BE has been proposed by the Substance Abuse and Mental Health Services Administration for federally-mandated workplace drug testing with patches worn for 7-14 days. In this study patches were only worn for 15 hours. It should be noted, however, that EME was detected more frequently and at higher concentrations than BE.

Keywords: Cocaine, Sweat, Controlled Cocaine Administration

Scientific Session Abstracts:

Postmortem Toxicology

NEW GENERATIONS OF ANTIDEPRESANTS IN FATAL INTOXICATIONS

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New generations of antidepresants are widely used for treatment of various types of depressive and other disorders. These drugs have a relatively low toxicity, and fatalitiesexclusively from these newer drugs are uncommon.

The authors describe six cases of fatal intoxications with a combination of some newer antidepresant with other drugs. Although these newer antidepresants are relatively safe, in combination with other drugs (especially with psychoactive drugs) they can cause death.

There drugs were isolated from biological specimes using either a diethyl ether extraction for qualitative analysis or by solid phase extraction for quantitative analysis.

Detection of drugs in biological specimes (urine, gastric contents, liver, kidney) was performed by thin layer chromatography/colour reactions (TLC/CR) and confirmed by gas chromatography/mass spectrometry method (GC/MS). Concentrations of drugs in postmortem bloods (subclavian artery blood) were determined using GC/MS method.

The results are presented in the table:

case	gender/age	antidepressant	blood lev.	other drugs	blood lev.
1.	F/41	citalopram	0.80	Promethazine	10.20
2.	F/47	moclobemide	18.20	Promethazine	7.44
3.	M/50	mianserin	1.33	Levomepromazine	0.51
				Dosulepin	2.76
4.	M/38	moclobemide	32.00	Promethazine	19.00
				Alcohol	1.88
5.	M/51	citalopram	1.24	Levomepromazine	0.04
				ketamine	NQ
6.	M/37	citalopram	2.39	Morphine	2.52

blood (subclavian artery) level: mg/l - drugs, g/kg - alcohol
NQ - no quantification

Keywords: new antidepresants, overdose, GCMS

IT'S ONLY JUST BEGUN A POSTMORTEM TISSUE DISTRIBUTION OF STRATTERA® OR ATOMOXETINE IN TWO FATALITIES

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Strattera® or Atomoxetine is a selective norephinephrine reuptake inhibitor approved by the Food and Drug Administration in December 2002. It is considered the first non-stimulant treatment of attention deficit hyperactivity (ADHD) in both children and adults. Atomoxetine is prescribed in 10, 18, 25, 40, and 60-mg dose capsules. Since Strattera® has entered the market, the Los Angeles County Dept. of Coroner Toxicology Laboratory has only encountered two cases where atomoxetine was detected. Presented are the case histories, cause and manner of death, the postmortem tissue distribution of atomoxetine, all designed to aid the forensic toxicologist with the interpretation of their own casework.

The analysis of atomoxetine from postmortem specimens consisted of a basic, liquid/liquid (n-butylchloride) extraction procedure with screening and quantitation by GC/NPD. Linearity was achieved from 0.05 to 5.0 mg/L with the limit of quantitation at 0.05 mg/L. Confirmation of atomoxetine was determined using a GC/MS by comparison with an analytical standard.

The tissue distribution of atomoxetine was as follows:

	Atomoxetine (mg/L or mg/kg)						
	Chest Blood	Femoral Blood	Vitreous	Liver	Gastric	Bile	Urine
Case No. 1	0.08	0.04	---	---	---	---	0.16
Case No. 2	1.3	0.23	0.34	4.2	1.2 or 0.17 mg total	1.5	0.62

It's the author's belief that these are one of the first cases of atomoxetine being detected and reported from postmortem specimens.

Keywords: Atomoxetine, Tissue Distribution, Postmortem

POSTMORTEM REDISTRIBUTION OF THE ENANTIOMERS OF CITALOPRAM AND ITS METABOLITES IN A RAT MODEL

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Enantioselective drug analysis is used to study the variation in the disposition of the enantiomers of racemic drugs. This type of analysis may give additional information in interpreting forensic toxicological results, provided that the postmortem redistribution is of the same magnitude for the enantiomers. One example of a racemic drug is the widely used antidepressant citalopram (CIT) that belongs to the selective serotonin reuptake inhibitors (SSRIs). Different therapeutic properties have been shown for the CIT enantiomers, and the S-(+)-enantiomer is responsible for the clinically relevant effects. Recent data indicate that the R-(–)-enantiomer instead counteracts the SSRI effect that is produced by the S-(+)-enantiomer. The major CIT metabolites, demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT), are less potent than the parent compound with regard to SSRI properties. In recent years, several reports on CIT concentrations in human postmortem cases have become available. However, only limited data are available describing postmortem concentrations of the separate enantiomers of CIT and metabolites. There is a general awareness that the concentration of a drug in an autopsy blood sample may not necessarily reflect the in vivo concentration just before death, and this difference is explained by postmortem drug redistribution. Thus, in order to accurately interpret the postmortem concentrations of CIT a general understanding of the changes in the enantiomer concentrations after death is required. Hence, the aim of the present study was to investigate if postmortem redistribution of the enantiomers of CIT, DCIT and DDCIT occurs in an experimental rat model after three different dosing procedures with racemic CIT. Two rat groups underwent chronic administration (20 mg/kg daily) using subcutaneously (s.c.) implanted osmotic pumps. After 10 days, one of these groups received an acute-on-chronic drug challenge by a single s.c. injection of 100 mg/kg. The third group received the single 100 mg/kg dose only. Heart blood and brain samples were collected antemortem and 1, 3 or 24 h postmortem after storage in room temperature. In an additional experiment, we examined the role of the lungs as a reservoir of postmortem drug release and if the early-phase postmortem redistribution was different in room temperature (21°C) as compared with a cold environment (4°C). The samples were analyzed with an enantioselective HPLC method with fluorescence detection. Increased postmortem blood drug and metabolite concentrations compared to corresponding antemortem concentrations were observed in all groups ($p<0.05$ to $p<0.001$). At 24 h after death, the ratios between postmortem and antemortem blood concentrations were around 3-4 for CIT as well as for the metabolites. In the brain, no major differences in any postmortem drug and metabolite concentrations were observed. Refrigeration at 4°C did not prevent, but significantly reduced the postmortem increase in heart blood CIT levels as compared to the concentrations in the rats stored at 21°C ($p<0.05$). The lung drug concentrations were lower postmortem than antemortem ($p<0.05$). The enantiomeric (S/R) concentrations ratios of CIT and metabolites in blood, brain and lungs were of similar magnitude before and after death. The parent drug to metabolite (P/M) ratios for CIT/DCIT were unchanged after death. In conclusion, this experimental study shows that heart blood CIT and metabolite levels increase rapidly after death, but that the S/R ratios and the P/M ratios remain unchanged, and thus still may be used in the interpretation of postmortem toxicological results. Further, a fall in postmortem CIT concentrations in the lungs was observed, indicating that the lungs seemed to represent the major source of drug release during early-phase postmortem redistribution.

Keywords: citalopram, postmortem redistribution, rat

INFLUENCE OF PUTREFACTION AND WATERY CIRCUMSTANCES ON THE CONCENTRATIONS OF ACONITINE IN TISSUES

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Aconitium plants (Ranunculaceae) are showy herbaceous flowering perennials that are widely distributed in the mountainous or cooler parts of northern temperate zones. They contain highly toxic *Aconitum* alkaloids such as aconitine, mesaconitine, hypaconitine and jesaconitine in the root, leaf and stem. Because of their high toxicity, *Aconitum* alkaloids have been used in suicides and homicides. In such cases, quantitative analysis of *Aconitum* alkaloids in the body fluids and tissues is necessary to determine the cause of death. For correct diagnosis of poisoning in cases where only putrefied material is available, it is necessary to investigate the influence of putrefaction on the concentrations of *Aconitum* alkaloids in body tissues. Moreover, we sometimes encounter cases of putrefied cadavers floating in the water, where toxicological analysis is necessary. Although the rate of non-enzymatic hydrolysis of aconitine dissolved in phosphate buffer has been studied, no reports are available on postmortem changes of *Aconitum* alkaloids in putrefied tissue. Therefore, we performed animal experiments to determine the influence of putrefaction over a 4-week period on the concentrations of *Aconitum* alkaloids in body tissues after oral administration of aconitine; one group of animals has been kept in the water for putrefaction, as a model of putrefied humans found in the water. ICR mice were divided into a whole-body storage group and an excised-organ storage group. Aconitine was administered orally at a dose of 4 mg/kg body weight to the mice, which were sacrificed within 35 min; for the whole-body storage group, each cadaver of the animals was placed in a bottle containing 750 ml water, followed by incubation at 20 or 35 °C for 1, 3, 7, 14 and 28 days. For the excised-organ storage group, the liver and brain were separately incubated without any water at 20 °C for the same intervals. The concentrations of aconitine and its hydrolysis products were measured by gas chromatography-mass spectrometry in the selected ion monitoring mode. In the whole-body storage group, the concentration of aconitine markedly increased in the liver and brain, showing postmortem diffusion of aconitine from the stomach. Concentrations of benzoylaconine and aconine also increased in the liver; but when expressed as ratios of the amounts of these substances to the total amounts of alkaloids, the ratios of aconitine gradually decreased, while those of its hydrolysis products increased. In the excised-organ storage group, no increase of aconitine concentration was observed; while benzoylaconine in the liver increased significantly on days 3 and 7. Only small amounts of aconitine were detected in the bottle water. The above results show that aconitine changes through hydrolysis and postmortem diffusion. But aconitine can be detected in the liver even on day 28. Our results of the whole-body storage group suggest that the postmortem diffusion of aconitine from the stomach to the brain *via* tissues and body fluids may occur in watery circumstances. Caution should be taken against the postmortem diffusion of aconitine for victims found in the water.

Keywords: Aconitine, Putrefaction, Postmortem diffusion

POSTMORTEM DETERMINATION OF SILDENAFIL IN BLOOD

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Sildenafil is sold as a remedy for an oral therapy for erectile dysfunction by the name of Viagra™. It is an inhibitor of cGMP phosphodiesterase and has a potential for cardiac risk during sexual activity in patients with preexisting cardiovascular disease. Therefore, patients who have cardiovascular problems should not use sildenafil citrate.

There have been 10 sildenafil related deaths in Korea. All were males in their late thirties to sixties. To investigate the cause of death, the gastric contents and blood underwent toxicological screening. To 1ml blood 50□ of 10□/□ tadanafil (Int.std) were added. Blood samples were purified by solid phase extraction using a Bond Elut-Certify column (Varian®). Blood was loaded into a cartridge that was pre-washed/conditioned with methanol and 0.1M phosphate buffer. The elution was performed with ethyl acetate:ammonia (98:2). After the solid phase extraction of blood, sildenafil was analyzed with high performance liquid chromatography with photodiode array detector (HPLC/PDA). The mobile phase consisted of 0.05M triethylamine-phosphate (pH 3.0), methanol and acetonitrile (580:250:170,v/v). Postmortem bloods were analyzed at a flow rate of 1.0ml/min with a detection wavelength of 290nm.

Standard curve of spiked sildenafil into blank blood was linear with a correlation coefficient of 0.998. The limit of detection and the limit of quantification for sildenafil were 0.02 μg/mL and 0.1μg/mL, respectively. The relative recovery was 99.4%.

The concentration range of sildenafil of postmortem blood was 0.00-0.70 μg/mL (mean 0.166±0.067) in all ten cases. In case 6, the blood concentration of sildenafil was below the limit of quantification (0.1 μg/mL), however, sildenafil was confirmed in the gastric contents. In case 1, 2 and 5, the victims had a medical history of cardiac disease.

Key words: Sildenafil; postmortem blood concentration, HPLC/PDA

TWO CASES INVOLVING CLOMIPRAMINE INTOXICATION

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Clomipramine and its active metabolite norclomipramine were identified and quantitated in multiple tissues recovered from two postmortem cases using liquid chromatography/mass spectrometry (LC/MS). The LC/MS method afforded the consistent chromatography needed to accurately determine both the parent drug and the more polar metabolite while simultaneously providing the specificity associated with mass spectral data.

In the first case a 23-year-old female with a history of depression presented to her attending pulmonologist with a respiratory tract infection. She was diagnosed with pneumonia upon admission to the hospital. The patient was placed on antibiotics and oxygen and appeared stable. Within twelve hours the patient became confused and anxious. She began to have difficulty breathing and subsequently coded and expired despite resuscitative efforts. An autopsy was conducted and tissues were submitted for toxicological analysis. Clomipramine and norclomipramine was detected in the following concentrations: Heart blood; 1.39 mg/L and 2.64 mg/L, Brain; 8.02 mg/kg and 26.74 mg/kg, Liver; 14.25 mg/kg and 41.12 mg/kg, Urine; 0.48 mg/L and 1.15 mg/L and Gastric Contents 3.99 mg/total and 0.61 mg/total.

Case #2 was a 56-year-old female with a history of depression who had previously attempted suicide with prescription painkillers. The evening prior to her death, the decedent had no complaints and retired for the night. The following morning she was found dead in her bed. Autopsy revealed evidence of medication around her mouth and in her gastric contents. No significant natural disease was present and toxicology was requested to assess the possibility of an overdose. In this case clomipramine and norclomipramine was detected in the following amounts: Femoral blood; 0.70 mg/L and 0.66 mg/L, Pulmonary artery blood; 1.00 mg/L and 1.11 mg/L Brain; 4.86 mg/kg and 7.43 mg/kg, Liver; 11.68 mg/kg and 20.76 mg/kg, Gastric 21.20 mg/total and 1.34 mg/total and Small Intestinal contents; not detected and 0.10 mg/total. The other significant finding was the presence of hydrocodone in femoral blood at a concentration of 0.13 mg/L.

In both cases the determination of clomipramine and metabolite in the brain samples was considered especially useful for assessing toxicity. The author's found the brain to be helpful for several reasons. First, although the blood results were potentially toxic, the liver results were not conclusively indicative of an overdose. Patients that have undergone long-term tricyclics antidepressant (TCA) therapy can sequester substantial amounts of parent drug and metabolite in liver, a particular concern because the TCA's may exhibit postmortem redistribution. In some cases of TCA overdose the liver results are unmistakable (i.e. clomipramine concentrations exceeding 200 mg/kg after acute poisoning). In the cases detailed in this report the liver results were considered insufficient to confidently establish fatal toxicity. Blood specimens (especially central) were viewed critically while interpreting results due to the concern of possible postmortem changes. The use of brain was advantageous because concentration changes are unlikely to occur in brain tissue due to postmortem redistribution. In these cases the analysis of brain provided further insight with respect to toxicity by establishing the concentrations of clomipramine and norclomipramine at the site of action without complications arising from postmortem redistribution.

This communication supplements the database on clomipramine and norclomipramine by providing quantitative determinations of both parent drug and metabolite in multiple tissues. A literature search revealed a paucity of data on clomipramine and norclomipramine levels in general and an absence of documented brain levels. When conducting these postmortem investigations, the inclusion of brain determinations provided valuable information in assessing the magnitude of toxicity in these cases involving clomipramine and its active metabolite norclomipramine.

Keywords: clomipramine, postmortem redistribution, tricyclic antidepressants

A COMBINED DRUG INTOXICATION INVOLVING METAXALONE (SKELAXIN®)

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We present a case of a 24-year old female who was found dead on her bedroom floor, in a secure apartment. She had a history of anxiety and had been prescribed antidepressants for several years. A scene investigation revealed empty beer cans and numerous prescription and over-the-counter medications. Prescribed medications included Lexapro® (citalopram), Skelaxin® (metaxalone), Ritalin® (methylphenidate), and Restoril® (temazepam). An autopsy was performed and the findings were unremarkable. Specimens were submitted for a full toxicological analysis, including an alcohol analysis by headspace gas chromatography with flame ionization detection; a screen for drugs of abuse and several prescription drug classes using an enzyme-linked immunosorbent assay technique (ELISA); and a screen for basic compounds using gas chromatography-mass spectrometry (GC-MS). Positive findings were confirmed and quantitated using GC-MS. The following drug concentrations were measured in postmortem blood: metaxalone 38 mg/L, citalopram 0.49 mg/L, diphenhydramine 0.10 mg/L and ethanol 0.02 g/100 mL. Methylphenidate and temazepam were not detected. Further investigation revealed the following prescription history for the decedent prior to her death:

Drug (mg per tablet)	Daily Dose	# Prescribed	# Remaining	Prescription filled
Skelaxin (400 mg)	400-800, tid	100 tablets	66 tablets	5 days prior to death
Ritalin (10 mg)	60 mg	180	0	2 weeks prior to death
Restoril (15 mg)	15-30 mg	60	0	2 weeks prior to death
Lexapro (200 mg)	200 mg	30	5	5 weeks prior to death

Metaxalone is a centrally acting muscle relaxant prescribed for the relief of pain associated with acute, musculoskeletal conditions. The recommended dose for adults is 2 x 400 mg tablets, three to four times a day. The circumstances and drug results in this case were similar to those reported in the only other metaxalone-related fatality cited in the literature: a 29-year old female with a history of depression had postmortem blood metaxalone and citalopram concentrations of 39 mg/L and 0.77 mg/L, respectively. In the present study, the cause of death was determined to be due to "*combined effects of metaxalone, citalopram, diphenhydramine and ethanol*", and the manner of death was "*undetermined*". A discussion of the case circumstances, the toxicology findings and the pharmacology of metaxalone will be presented.

Keywords: Metaxalone, Skelaxin, Intoxication

A CASE OF FATAL OVERDOSE WITH LABETALOL

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Labetalol (Normodyne®, Trandate®) is an alpha and beta-adrenergic and beta-adrenergic receptor antagonist used in the treatment of hypertension. A typical dose of labetalol ranges from 200 – 400 mg/day however some individuals may require up to 2100 mg/day. Symptoms of labetalol overdose are excessive hypotension and bradycardia. There is very limited information available regarding labetalol concentrations and toxicity and no reports where the cause of death has been attributed solely to a labetalol overdose. This study describes a case where the cause of death was attributed to an acute overdose of labetalol.

A 79-year-old female was admitted to hospital for high blood pressure and chest pain. She was stabilized and after remaining in hospital for 7 days was being prepared for discharge. At this time she was observed to have swallowed numerous tablets by another patient. She subsequently became hypotensive and bradycardic, and resuscitation attempts were unsuccessful. A blood sample taken during the attempted resuscitation was tested at the hospital and the drug screen showed that labetalol was significantly higher than the therapeutic range. Empty prescription bottles of labetalol, lorazepam and furosemide (medications she brought with her to the hospital) were found at the scene, however the actual number of tablets taken is not known. There were no other significant findings in the hospital drug screen except for the elevated labetalol concentration.

A postmortem was conducted and toxicology testing was performed on a heart blood sample. Labetalol was measured by high-pressure liquid chromatography (HPLC) and the detected concentration was 15 mg/L. Lorazepam was measured by mass spectrometry (MS) and was detected at a concentration of 73 ng/mL. In addition to specific testing for labetalol and lorazepam, a general basic drug screen using gas chromatography and mass spectrometry (GC and GC/MS) was performed and traces of sertraline and metoprolol were detected. This laboratory does not have a method for the analysis of furosemide.

Therapeutic concentrations of labetalol in serum have been shown to range from 0.02 – 0.25 mg/L, and levels over 0.5 mg/L are considered to be toxic. In one case report, a woman who suffered renal failure following an acute ingestion of labetalol (16g) had a serum concentration of 29 mg/mL. The concentration of labetalol in this case is approximately 60 times higher than the therapeutic levels. The autopsy showed signs of chronic hypertension but no gross or microscopic cause of death. In the absence of other significant drug findings the cause of death was reported as acute labetalol intoxication.

Key words: labetalol, fatality, overdose

DETERMINATION OF OXCARBAZEPINE AND ITS METABOLITES IN POSTMORTEM SPECIMENS INCLUDING HAIR BY MEANS OF LIQUID CHROMATOGRAPHY WITH MASS DETECTION (HPLC/APCI/MS) IN AN EPILEPTIC PATIENT AFTER A FATAL DRUG-POISONING

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One use of hair analysis in forensic toxicology is the documentation of prior drug administration. This is illustrated in a suicidal death of a 58-year-old epileptic patient who was treated with oxcarbazepine and probably with levomepromazine.

The toxicological analysis carried out by HPLC/APCI/MS included also the hair (6cm length) besides of postmortem blood and liver samples. The method was validated for oxcarbazepine (OXCBZ) and its two metabolites, 10-hydroxycarbazepine (CBZ-10OH) and trans-diol-carbazepine (CBZ-diOH) in various biological matrices.

The toxicological findings are as follows:

	Hair			Autopsy	
	Segment (cm) I	Segment (2 cm) II	Segment (2 cm) III	Blood	Liver
	Concentration (μg/g)				
Oxcarbazepine	3.9	10.4	13.0	0.79	n.d.
CBZ-10OH	18.4 4.7*	53.9 5.1*	105.9 8.1*	13.96 17.6*	15,28 -
CBZ-diOH	0.5 0.12*	1.2 0.12*	3.0 0.23*	0.23 0.29*	n.d -
Levomepromazine	-	-	-	1.96	30,25

*Relative concentration metabolite / prekursor
n.d. – not detected

The analysis of the postmortem blood indicated oxcarbazepine and its two main metabolites were present at therapeutic concentrations; levomepromazine was detected at a fatal concentration. In three 2-cm segments of hair, oxcarbazepine and its two metabolites were detected; however, levomepromazine was not detected in this specimen. A complex chemical-toxicological investigation confirmed the information that the decedent. was an epileptic patient and was treated with oxcarbazepine for at least 6 months before death. In addition, he took a toxic dose of levomepromazine in order to commit suicide. The analysis revealed differences between the concentration levels of oxcarbazepine and its active metabolite CBZ-10OH in postmortem specimens and hair, suggesting different mechanisms of penetration of certain metabolites and their precursors into these materials.

Keywords: oxcarbazepine, hair, LC/APCI/MS

STABILITY OF SULFONYLUREAS IN STORED POSTMORTEM BLOOD SPECIMENS AND WATER STANDARDS

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Stability of three commonly prescribed sulfonylureas (glipizide, chlopropamide and glibenclamide) in postmortem blood and water stored at –20, 4, and 25°C were evaluated over a 1-year period. The drugs were analyzed simultaneous by utilizing solid phase extraction techniques and were identified and quantified by high performance liquid chromatography. The data showed that, significant loss of glibenclamide (GB) was noticed at second and third week of storage at 25 and 4°C respectively. The decrease was gradual but no clear pattern could be established. At the end of the 1-year storage period at either 4 or 25°C, the level of GB was about 37% and 46% of its initial value in aqueous solution and postmortem blood samples respectively. There was no significant loss of both glipizide and chlopropamide in all stored samples over the first 6-month period. After that time a significant decrease in their levels were noticed, and at the end of the 1-year of storage concentrations ranged from 67.5 to 84.1% of their initial values. Storage at -20°C showed the least loss of drug concentration. Authentic samples of these drugs showed similar results. This study supports the importance of paying careful attention to using prepared aqueous drug standards even when stored in cold conditions. In order to obtain reliable results of drug analysis and drug stability in biological samples, such studies should be considered after prolonged sample storage time. The best storage temperature to keep aqueous standards and blood samples for the investigated drugs with sufficient stability was found to be at -20°C.

Keywords: Stability, Sulfonylurea, Blood.

DISTRIBUTION OF QUETIAPINE IN NINE POSTMORTEM CASES

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Quetiapine (Seroquel ®) is an antipsychotic drug approved by US FDA in September 1997 for the treatment of schizophrenia. It is classified as a dibenzothiazepine and structurally related to clozapine. The molecular formula is $C_{21}H_{25}N_3O_2S$ with a molecular weight of 383.6. It is believed to exert its pharmacological effect through antagonism of sertonergic (5HT2) and dopaminergic (D2) receptors in the brain. Quetiapine is extensively metabolized, but the pharmacological effect is primarily attributed to parent drug. It is rapidly absorbed after oral administration, reaching peak plasma concentrations in 1.5 h. The elimination half life is 6 h. Peak serum quetiapine concentrations range from 0.17 – 0.37 mg/L following a single oral 75 mg dose to 0.19 – 0.63 mg/L following a 450 mg dose.

Nine postmortem quetiapine cases identified by the Virginia Division of Forensic Science toxicology laboratories during 2003 are presented. Quetiapine was identified by full scan electron impact gas chromatography-mass spectrometry (GCMS) following a basic drug extraction from blood. Quantitation was performed using a basic solid-phase extraction followed by the addition of BSTFA and selected ion monitoring GCMS. The following mean tissue concentrations and ranges were determined: heart blood 18.9 mg/L (0.53 – 76 mg/L, n = 4), peripheral blood 11.5 mg/L (0.46 – 37 mg/L, n = 6), urine 16.4 mg/L (1.9 - 37 mg/L, n = 6), liver 123 mg/Kg (7.2 – 510 mg/Kg, n = 9), and gastric 48.2 g/L (15-130 g/L, n = 4). Medical examiners certified the cause of death in eight of the nine cases as combined drug toxicity. In one case the cause of death was quetiapine toxicity and the manner of death was suicide. The quetiapine concentrations in this case were 15 mg/L in antemortem blood, 18 mg/L in peripheral postmortem blood, and 89 mg/Kg in liver. Only one case contained both heart and peripheral blood (7.0mg/L and 3.4 mg/L respectively). The heart/peripheral blood ratio was 2.0 suggesting postmortem redistribution.

Keywords: quetiapine, postmortem, distribution

DETERMINATION OF ATRACTYLOSIDE IN *CALLILEPIS LAUREOLA* USING SOLID-PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY – ATMOSPHERIC PRESSURE IONISATION MASS SPECTROMETRY

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A selective analytical method based on high-performance liquid chromatography, combined with atmospheric pressure ionisation mass spectrometry, was developed for the detection of atractyloside. The tuberous root of the plant *Callilepis laureola* is used in traditional medicine by the Zulu and Xhosa people of South Africa and it is well known by the Zulu name *impila*, which means 'health'. However, *C. laureola* has also been implicated in the death of numerous patients who used medication prepared from *impila* in which atractyloside, the dipotassium salt of a sulfonated kaurene glycoside, was identified as the toxic principle. This compound, together with carboxyatractyloside, was also identified as the toxic principle of *Atractylis gummifera* L. (Asteraceae), a plant linked to human fatalities in Mediterranean countries since ancient times.

The analysis was performed on an Xterra Phenyl column utilising a gradient elution profile and a mobile phase consisting of 10 mM aqueous ammonium acetate buffer-MeOH-acetonitrile. The developed method showed a good linearity for the calibration curve spanning the 10 ng/mL to 1 μg/mL (r^2 = 0.997) range. The limit of detection and limit of quantitation were determined and found to be 100 pg/mL and 10 ng/mL, respectively. The method was successfully applied to the analysis of *C. laureola* tuber, herbal medicine mixtures and viscera samples (stomach, stomach contents, liver, kidney, blood, urine, bile) for the presence of atractyloside.

Keywords: Atractyloside, *Callilepis laureola*, HPLC-MS

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MIRTAZAPINE-RELATED DEATHS

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Mirtazapine is a novel antidepressant drug which has a pharmacological action on both noradrenergic and serotonergic systems. The drug has been on the US and European markets for about 10 years, but relatively few deaths associated with mirtazapine have been reported in the literature. Post-mortem blood mirtazapine concentrations of up to 0.3 mg/L have been reported in cases where the drug is not implicated in the cause of death. There is also evidence to show that the drug undergoes post-mortem redistribution, which can make interpretation of findings more difficult.

Relatively few deaths reported in UK have noted the presence of mirtazapine. However, in the last few years (2001-2003) the Laboratory has investigated a total of 8 cases (5 male, 3 female) aged between 17 and 77 years where mirtazapine has been found to be present in significant concentrations, but generally in association with other drugs. Post-mortem blood mirtazapine and its desmethyl metabolite (normirtazapine) have been measured using liquid chromatography with U.V-diode array detection. In addition, extensive screening of blood and other fluids was undertaken to determine the presence of other drugs and alcohol.

Details of the 8 cases investigated are shown in the Table below. In 7 of the 8 cases blood was taken from a peripheral site at post-mortem. Post-mortem blood mirtazapine concentrations ranged between 1.0 – 4.4 mg/L (mean 2.1 mg/L) The ratio of mirtazapine to normirtazapine concentrations ranged between 1.9 and 20.

Age	*Sex*	*Site of Collect*	*Mirtaz (mg/L)*	*Normirtaz (mg/L)*	*Mirt/Normirtaz Ratio*	*Other drugs present and (concentrations mg/L)*
75	M	Femoral	2.2	0.3	7.3	Temazepam, (2.2), venlafaxine (3.6). Found dead at home
46	M	Femoral	2.0	<0.1	>20	Temazepam (1.1) ethanol (214 mg/dL). Found dead at home
60	M	Unknown	1.6	0.8	2.0	No other drugs detected. Found dead in river
56	M	Femoral	1.0	0.3	3.3	Diazepam (1.5), Sildenafil (trace), alcohol (72 mg/dL). Found dead at home
77	M	Femoral	2.1	1.1	1.9	Zopiclone (1.6). Found dead in bed
51	F	Femoral	2.5	0.4	6.7	Zopiclone (1.7), Lorazepam (0.4), chlorpromazine (trace), alcohol (54 mg/dL). Found dead in bath
21	F	Common Iliac	1.3	0.3	5.0	Dextropropoxyphene (2.4), Paracetamol (124), Venlafaxine (1.4) Zopidem (trace). Found dead at home
17	F	Femoral	4.4	0.7	6.1	Diltiazem (4.2), paracetamol (70), Zopiclone (trace). Found dead at home with suicide note

In conclusion, mirtazapine appears to be more commonly seen in recent post-mortem cases, probably due to prescribing trends. In all but one of the cases, other drugs or alcohol were present. However, mirtazapine overdosage appeared to be a significant factor in at least half of the deaths reported and were associated with a parent blood mirtazapine concentration >2.0 mg/L and a ratio of mirtazapine to normirtazapine >2.

Keywords: blood, mirtazapine, postmortem

P14

TWO FATAL INTOXICATION CASES WITH IMIDACLOPRID: LC-MS ANALYSIS

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Aim: Imidacloprid [1-(6-chloro-3pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] is a new and potent nitromethylene insecticide with high insecticidal activity at very low application rates. It is the first highly effective insecticide for which the mode of action has been found to deviate from the almost complete and irreversible blocking of the postsynaptic nicotinergic acetylcholine receptors. The authors present two fatal cases due to this pesticide, in two males, 33 and 66 years old.

Methods: An LC-MS with electrospray method is described for measuring imidacloprid and its metabolite in postmortem samples. In the chromatographic separation, a reverse-phase column C_{18} (2.1x150mm, 3.5µm) was used and the mobile phase composed with acetonitrile and 0.1 % formic acid (15:85), at a 0.25 ml/min flow rate. The samples were prepared by a liquid-liquid extraction procedure with dichloromethane.

Results: Calibration curves for imidacloprid were performed in acetonitrile in blood and urine, achieving linearity between 0.2µg/ml and 15µg/ml. The mean recovery was 86%, coefficient of variation was ±5.9% and the detection limit 2.0ng/ml. Quantitative results were obtained for all postmortem matrices available of the two fatal cases: blood, urine, stomach contents, liver and kidney. The concentrations found ranged between 0.29µg/ml (urine) and 350µg/ml (stomach contents). Postmortem blood concentrations found were 12.5µg/ml and 2.05µg/ml.

Conclusions: The authors developed a sensible and validated method to detect and quantify imidacloprid in postmortem samples and emphasize the lack or even absence of literature with fatal cases related to this insecticide.

Keywords: Imidacloprid, fatal intoxication, LC-MS

LEVELS OF LEVETIRACETAM IN POSTMORTEM BLOOD

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Levetiracetam (LEV) is a recently introduced anticonvulsant agent and little information is available to assist in the interpretation of levels seen in post-mortem cases. Our objective is to report our observations from three Medical Examiner cases in which LEV was detected.

LEV is said to have "ideal" pharmacokinetic characteristics for clinical application. It is well absorbed after oral administration and is distributed approximately into body water. It is not appreciably bound to plasma protein and its apparent plasma elimination half-life is near to 6 hours.

LEV was extracted from acidified blood into dichloromethane and was assayed by gas chromatography / mass spectrometry using hexobarbital as internal standard. The retention factor, relative to hexobarbital, was 0.79 on a HP-5 column (initial temp, 50 °C; time 1, 1 min; ramp 1, 50 °C /min to 100 °C; ramp 2, 20 °C/min to 285 °C). The electron impact mass spectrum contained an intense ion at m/z 126, minor ions at m/z 170 (m*), 98 and 69.

Case summaries and levels of LEV in postmortem blood are reported in Table 1. The data suggest that postmortem levels of LEV near 60 mg/L may be within the normal therapeutic range, and might be encountered, without toxicological consequence. A lower level, near 10 mg/L as seen in case number 3, may be sub-therapeutic.

Table 1: Levels of Levetiracetam (LEV) in postmortem blood, Case 1, femoral. Cases 2 and 3, source not indicated

Case #	Age	Sex	LEV (mg/L)	Other drugs (mg/L blood)	Cause of Death	Manner
1	22	M	60	None detected	Idiopathic epilepsy, cerebral sclerosis	Natural
2	58	M	61	Hydrocodone, 0.25 Metoprolol, 1.4 Amitriptyline, 0.38 Nortriptyline, 0.57 Paroxetine, 0.33 Trazadone, 0.93 Phenobarbital, < 10	Hypertensive cardiovascular disease Post -cerebral vascular infarct seizure disorder	Natural
3	35	M	11	None detected	Drowning, Seizure due to remote leptomeningitis	Accident

Keywords: Levetiracetam, blood, postmortem

OPTIMIZING AN AUTOMATED SOLID-PHASE EXTRACTION PROCEDURE FOR POSTMORTEM TISSUE SAMPLES

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Quality assurance regulations in forensic laboratories, efforts to reduce systematic errors, costs, time spent per case as well as efforts to improve technician-safety all lead to the consideration of automated procedures for sample extraction. In contrast to liquid-liquid extraction, solid-phase extraction (SPE) can be more easily automated by incorporating robotics – improving the sample quality in respect to the analyte yield and increasing consistency and laboratory productivity.

Today, automated SPE procedures are widely used for the extraction of body fluids such as urine, serum, plasma and whole blood. In postmortem forensic toxicology, however, these specimens are not always available and, with regard to the investigation of the cause of death, human tissue samples - especially brain - can be highly important.
Drummer and Gerostamoulos pointed out that "... there is little evidence that extraction efficiencies of drugs from solid tissues are likely to be much worse than with fluid specimens if suitable precautions are taken. These include a suitably fluid homogenate prepared from the solid tissue with sufficient water or buffer" [1].

In an automated SPE procedure published by our working group, a crude extract from postmortem tissue samples could be achieved, avoiding protein precipitation and the resulting loss of analytes by adsorption or occlusion.
In respect to the problem that tissue homogenates often do not easily pass through tightly-packed cartridges, it could be shown that - after homogenization and dilution with large volumes of buffer solution – the resulting colloidal solution could be directly applied to the polymeric sorbent [2].

For routine solid-phase extraction of tissue samples, several adaptations of the automated system ASPEC™XL, operated under 735-sampler software V 5.1 for Win NT 4.0 (Gilson Inc., Middleton, WI, USA), were necessary.

- A large volume of sample had to be applied in order to dilute the sample and dissolve protein bonds.
- The upper frit of the extraction cartridge had to be removed to minimize the risk of clogging.
- A 5mL stainless steel sample loop for HPLC (Supelco, Bellefonte, PA, USA) was installed on the ASPEC XL to avoid adsorption of analytes to synthetic plastic materials (transfer tubing).
- Special cleaning routines with 2N NaOH and 2N HNO_3 were used to remove any precipitated proteins from the system.
- The sample was applied very slowly to make use of "micellar chromatography", thus avoiding the adsorption of macromolecular interferences with larger than pore-sized diameters to the polymeric sorbent.
- A pressure sensor was installed to compensate for inconsistent flow rates during loading, washing and eluting.

When postmortem samples of tissue have to be extracted by automated SPE, monitoring of the overall system pressure is essential. Pressure control allows for high-pressure release if clogging of the cartridge occurs, avoiding contamination of the working area with the infectious sample; the aborted sample is then replaced to the sample tube.

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Keywords: automation, solid-phase extraction (SPE), tissue samples

POSTMORTEM DISTRIBUTION OF TRAMADOL, AMITRIPTYLINE AND THEIR METABOLITES IN A SUICIDAL OVERDOSE

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A case report involving a 34 year old white male who was found dead at home by a roommate is presented. The decedent's history included a suicide attempt and pain as result of a motor vehicle accident occurring 5 months earlier. At the time of death, he was being treated with tramadol/acetaminophen, metaxalone, oxycodone and amitriptyline. The descendant's mother stated that he had been taking increasing amounts of pain medication in order to sleep at night.

Further investigation of this case was based on the fact that seven tissues were available for a fatal multi-drug ingestion concerning several analytes of interest, one of them being tramadol, which has limited information concerning tissue and metabolite concentrations. Toxicology tests yielded acetaminophen, oxycodone and cyclobenzaprine aortic blood concentrations of 140, 0.42, and 0.97 mg/L, respectively. Metaxalone and ibuprofen were detected at below therapeutic concentrations. The following matrices were analyzed for tramadol, n-desmethyltramadol, o-desmethyltramadol, amitriptyline and nortriptyline: aortic and iliac blood, urine, liver, vitreous humor, bile, brain, heart, kidney, lung, muscle and spleen. Analytes were quantitated by electron impact gas chromatography/mass spectrometry (GC/MS).

The following table contains the postmortem analyte concentrations obtained for each tissue analyzed in this study.

Matrix	Tramadol	N-Desmethyl Tramadol	O-Desmethyl Tramadol	Amitriptyline	Nortriptyline
Blood (aorta) mg/L	31.0	BQL*	0.901	5.79	1.49
Blood (iliac) mg/L	6.21	0.20	0.68	2.33	0.88
Liver (mg/kg)	14.0	BQL	2.47	82.3	29.1
Urine (mg/L)	55.3	1.45	8.27	27.1	6.63
Vitreous Humor (mg/L)	3.32	BQL	0.25	0.25	BQL
Bile (mg/L)	2.95	BQL	0.45	41.4	4.59
Brain (mg/kg)	4.62	BQL	0.73	24.3	9.29
Heart (mg/kg)	4.95	BQL	1.08	17.4	8.98
Kidney (mg/kg)	9.61	BQL	2.54	25.8	8.40
Lung (mg/kg)	87.0	BQL	1.15	134	22.2
Muscle (mg/kg)	22.0	BQL	BQL	22.6	2.47
Spleen (mg/kg)	29.1	BQL	2.61	28.8	16.9

* BQL- Below quantitation limit of 0.25 mg/L (mg/kg).

For all analytes, the parent-to-metabolite ratios support an acute toxicity with the parent concentrations being 5-10 times greater than its major metabolite. For amitriptyline and nortriptyline, the order of highest to lowest concentrations detected are lung, liver, bile, urine and other solid tissues (spleen, kidney, brain, muscle and heart), central and peripheral blood, and finally vitreous humor. On the other hand, tramadol and its metabolites demonstrate a similar concentration trend with the exception of central blood and liver. It has been suggested that tramadol does not sequester into liver tissues or demonstrate postmortem redistribution from drug-rich tissues. This case does not support or refute these observations since gastric contents were not collected and we cannot confirm the possibility of diffusion from the gastrointestinal tract to the central blood or timing of the ingestion of these drugs with respect to one another. There were no significant findings at autopsy; however, toxicology results supported a cause and manner of death resulting from suicidal mixed tramadol and amitriptyline toxicity. This case reports the tissue and fluid distribution of tramadol, amitriptyline, and their metabolites in an acutely fatal ingestion in an effort to document concentrations of these analytes in 12 matrices with respect to one another to assist toxicologists in difficult interpretations.

Keywords: postmortem distribution, tramadol, amitriptyline

SCREENING BY ELISA FOR CARISOPRODOL IN POST-MORTEM BLOOD

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Carisoprodol is an unscheduled substance widely prescribed in the USA to promote skeletal muscle relaxation. The use of carisoprodol is associated with CNS depression, which can lead to impairment in driving and other psychomotor activities. Therefore, it is very important to have a reliable method to test post-mortem blood sample for carisoprodol. Using Immunalysis Direct Elisa Kit and the PersonnalLAB™ as the analyzer we developed an application to screen carisoprodol in post-mortem blood. We tested a variety of samples using different sample volumes and cutoffs. Using 25uL as the sample volume and 500ng/mL as the cutoff, thirty-two samples were analyzed by ELISA v. GC/MS and GC/NPD. Ten were positive by both the immunoassay and the chromatographic methods. One sample screened between the negative control and the cutoff calibrator, and was found to contain 4.78 mg/mL of carisoprodol. The concentrations of carisoprodol in samples that screened positive ranged from 0.12 mg/L to 45.2 mg/L. These samples also contained meprobamate, ranging in concentration from 2.26 mg/L to 20.2 mg/L. None of the ELISA-negative samples contained carisoprodol at greater than 500 ng/mL. The twenty-one samples that were negative by both the ELISA and by the chromatographic methods contained a broad range of other drugs, including cocaine, benzoylecgonine, codeine, morphine, hydrocodone, methadone, amitriptyline, nortriptyline, diazepam, nordiazepam, temazepam, alprazolam, acetaminophen, bupropion and its metabolites, norchlorcyclizine, nicotine, cotinine, caffeine, olanzapine, fluoxetine, norfluoxetine, norpropoxyphene, doxylamine, tripolidine, and metoclopramide. A precision study was conducted by analyzing 10 replicates each of the negative calibrator, a 250 ng/mL control, the 500 ng/mL calibrator, and a positive control containing 1000 ng/mL carisoprodol. The OD's were tracked and the results were as follows: negative calibrator: 1.386 $\pm$ 0.051 (CV= 3.7%); 250 ng/mL control: 0.510 $\pm$ 0.015 (CV= 2.9%); 500 ng/mL calibrator: 0.440 $\pm$ 0.024 (CV= 5.4%); and 1000 ng/mL control: 0.410 $\pm$ 0.014 (CV= 3.4%).

Keywords: Carisoprodol; immunoassay; postmortem blood

EVALUATION OF DEBATED QUESTIONS: DO POSTMORTEM METHADONE CONCENTRATIONS OF ALTERNATIVE SPECIMENS HAVE INTERPRETIVE VALUE?

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Deaths due to methadone ingestion are escalating in many geographical locations nationwide. Often these deaths are the result of naïve users ingesting methadone either recreationally or as prescribed, however, their systems do not tolerate the dose taken. With the increased use of methadone, either as a recreational drug or to treat chronic pain, the population dying from methadone toxicity has radically changed over the last decade. Many factors contribute to the difficulty of interpreting methadone's role in a death. Some postmortem laboratories do not practice analyzing alternative matrices to interpret methadone's contribution to a death. In fact, limited publications discuss whether postmortem redistribution occurs and if so to what degree. Furthermore, some postmortem laboratories have traditionally ignored methadone liver concentrations based on the belief that their interpretative values are minimal. Moreover, overlapping therapeutic and toxic ranges make it difficult to interpret methadone's contribution to a death without having additional history, pathological findings (e.g. bronchopneumonia or perimortem aspiration), and/or dosing regime. Given these observations, the Office of the Chief Medical Examiner (NC-OCME) retrospectively reviewed all deaths in the State of North Carolina between 2001 and 2002 in which methadone was detected during toxicological analysis. This presentation evaluates 223 death investigations in which methadone was detected in several postmortem tissues and fluids to ascertain whether or not data from alternative specimens are more useful than originally thought.

After careful review of the history, pathology and toxicology findings, it was determined that 180 (80%) cases could be evaluated given the data obtained. Exclusion criterion included cases in which the prescription status was unknown or multiple drugs were detected in toxic concentrations. To assist in interpretation, these cases were divided into several groups based on their drug use pattern and the assigned cause of death. These groups are as follows: 1) methadone-related death in a methadone-prescribed/tolerant individual 2) methadone-related death in a methadone-not prescribed or -recently prescribed/ non-tolerant individual 3) methadone unrelated to death or indirectly contributed to death (i.e. controls). The following table presents data from representative observations of this study:

Group		Central Blood (mg/L)	Peripheral Blood (mg/L)	Central/ Peripheral Ratio	Liver (mg/kg)	Liver/ Central Ratio	Presence of Broncho-pneumonia	Presence of aspiration
1	mean	1.1	0.51	1.5	3.9	6.2		
(n= 46)	median	0.68	0.36	1.5	2.6	4.7	10 (21%)	3 (6.5%)
	range	0.1-9.9	0.07-1.9	0.41-2.3	0.44-17	0.72-19		
2	mean	0.49	0.34	1.8	2.5	7.5		
(n= 105)	median	0.38	0.35	1.2	2.2	6.3	42 (40%)	14 (13%)
	range	0.05-2.5	0.047-0.82	0.47-7.3	0.36-9.3	1.4-25		
3	mean	0.35	0.27	2.4	2.6	7.9		
(n= 29)	median	0.28	0.25	2.2	1.7	6.4	4 (13%)	2 (6.8%)
	range	0.05-1.3	0.03-0.72	1.2-3.8	0.19-27	1.3-28		

This investigation supports that postmortem redistribution of methadone can occur and may be significant in some cases. Liver concentrations when compared to central blood do not readily distinguish a methadone overdose from a methadone-unrelated death, especially if the dosing regime is unknown. Bronchopneumonia and perimortem aspiration, although they can support methadone ingestion, do not always occur (13-40% and 6-13%, respectively). These data suggest that while collection and analysis of blood from a peripheral source, in addition to review and consideration of pathological findings and drug use history, can improve the interpretation of toxicological data in methadone-related deaths, liver concentrations may have less interpretive value.

Keywords: Methadone, Interpretation, Postmortem Fluids and Tissues

A COMPARISON OF METHADONE, HYDROCODONE, AND OXYCODONE ASSOCIATED MORTALITY IN CUYAHOGA COUNTY, OHIO: 1998 – 2003

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Significant increases in methadone related deaths have been recently documented across the United States. Historically, the substantial rise in oxycodone associated mortality is well documented as opposed to hydrocodone, where a paucity of literature remains. In response to these reports, the authors investigated cases over a six-year period in which postmortem toxicological analyses revealed the presence of methadone, hydrocodone, and oxycodone in any matrix type. The study was designed to determine whether methadone associated mortality in Cuyahoga County reflected national trends and more specifically, to distinguish methadone trends from other commonly used opioid analgesics.

All records of decedents that were found to be positive for methadone, hydrocodone, and oxycodone from 1998-2003 were reviewed. Demographic information including age, sex, race, and location of residence was collected. The cause and manner of death was compiled and the cases were divided into drug overdoses and those cases where a positive result was determined to be an incidental finding. Overdoses as a result of only methadone, hydrocodone, and oxycodone were separated from polydrug overdoses. The data was calculated for each type of drug based on quantitative heart blood drug concentrations for comparison.

Review of 21,460 deaths in Cuyahoga County, from 1998-2003, revealed that a total of 55 decedents were positive for methadone and of these, 29% were ruled overdoses. 202 cases were found to be positive for hydrocodone in which, 28% were due to a lethal dose. Oxycodone was discovered in 190 decedents of which, 29% were caused by an overdose. Mortality caused by methadone, hydrocodone, and oxycodone increased 400%, 183%, and 366% respectively from 1998 to 2003.

Demographic data revealed 286 males and 161 females were positive for the studied opioids. 351 opioid positive decedents were white, as opposed to 96 black. Decedent ages ranged from 2 to 101 years. Caucasian males between the ages of 34 and 51 (mean = 42.5 ±2SD) were the demographic group predominantly positive for each of the three opioids. Decedents in which methadone was detected were shown to live within Cleveland city limits vs. the suburbs 3:1, as opposed to hydrocodone and oxycodone positive decedents who principally resided in the suburbs.

Heart blood methadone overdose concentrations ranged from 0.11-1.31 mg/L (mean=0.67 mg/L, n=19) Similarly, heart blood methadone incidental finding concentrations ranged from 0.08-4.26 mg/L (mean=0.76 mg/L, n=35). Lethal hydrocodone heart blood concentrations ranged from 0.01-1.66 mg/L (mean=0.29 mg/L, n=54). Incidental hydrocodone findings ranged from 0.01-2.56 mg/L (mean=0.11 mg/L, n=112). Oxycodone overdose concentrations ranged from 0.01-36.54 mg/L (mean=1.80 mg/L, n=48). Oxycodone positive concentrations that were determined to be incidental findings ranged from 0.01-1.78 mg/L (mean=0.31 mg/L, n=81).

During the study period, an increase was observed in the number of positive cases for all three opioid analgesics. In conflict with recent national data however, although the number of methadone positive cases increased from 4 cases in 1998 to 18 cases in 2003, this did not result in a substantial increase in methadone overdoses, 1 death in 1998 to 4 deaths in 2003. No methadone level of lethal toxicity was discernable by comparing decedents whose death was caused by methadone intoxication as opposed to, an incidental finding. In contrast, the mean lethal hydrocodone and oxycodone blood concentrations were definable, at two and five times greater than non-overdose mean blood concentrations, respectively. Methadone, hydrocodone, and oxycodone overdoses equally comprised 28-29% of cases in which these drugs were detected.

Keywords: Methadone, Oxycodone, Hydrocodone

"STUDENT ON ALPHA-METHYLTRYPTAMINE DISCOVERS THE SECRET OF THE UNIVERSE... AND DIES"

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Alpha-methyltryptamine (AMT) is a synthetic indole analog of amphetamine initially investigated as a monoamine oxidase inhibitor. In the 1960s, the Soviet Union marketed AMT as an antidepressant under the name of Indopan. During the same period, the Upjohn Company studied the alpha-ethylated analog for use as a commercial antidepressant known as Monase, but found it to be of little medicinal value. Although clinical use of AMT is obsolete today, recreational use has gained popularity due to its intense hallucinogenic properties lasting up to 16 hours. To illustrate recreational use of AMT in the 1960s, Alexander Shulgin in his book *Tihkal*, references the author Ken Kesey and his experiences with AMT and other hallucinogenic drugs.

Today, AMT is recognized as a powerful psychedelic drug among high school and college-aged men and women who may have experienced the effects of other hallucinogenic amphetamines. Its popularity is partly due to the legality and availability of AMT for purchase via the Internet prior to April 2003. Emergency designation of AMT as a Schedule 1 controlled substance by the Drug Enforcement Administration occurred shortly after the Miami-Dade Medical Examiner reported the death of a 22 year-old college student who ingested a large amount of AMT. Prior to death, the deceased advised his roommate that he was "taking hallucinating drugs" and as a result had "discovered the secret of the universe". The roommate reported that the deceased was shaking and sweating profusely. Approximately 12 hours later, the roommate discovered the deceased lying in bed unresponsive. An empty 1gram vial of AMT was recovered from the scene and sent to the Toxicology Laboratory. An autopsy by the Medical Examiner did not reveal any significant gross findings of any organs, and no evidence of traumatic injury.

Initial screening of urine by enzyme-multiplied immunoassay technique was positive for amphetamines, and the basic drug blood screen detected a small peak later identified by mass spectrometry as AMT. For quantitation, AMT was isolated using solid phase extraction, derivatized with pentafluoropropionic anhydride, and analyzed using gas chromatography/mass spectrometry. Ions monitored for AMT included m/z 276, 303, and 466. Quantitative analysis was based upon m/z 276 for AMT and m/z 306 for the internal standard 5-methoxy-alpha-methyltryptamine. A linear calibration curve from 50 to 500ng/mL was used to calculate the concentration of AMT in the samples and controls. Blood, tissue, and gastric specimens were diluted to bring the observed concentration within the limits of the standard curve. Matrix matched controls were extracted and analyzed with each run. Postmortem blood revealed 2.0mg/L, gastric contents 9.6mg total, liver 24.7mg/kg, and brain 7.8mg/kg.

Keywords: Alpha-methyltryptamine, 5-Methoxy-alpha-methyltryptamine, hallucinogens

TILL DEATH DO US PART: FENTANYL POISONING IN A HUSBAND AND WIFE

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One treatment modality for chronic pain is the application of fentanyl transdermal patches (Duragesic®). However, the transdermal therapeutic system, designed for the rate-controlled delivery of drug, is increasingly favored by members of the opioid-misusing/abusing population. Deliberate misuse or abuse may include cutaneous application of multiple patches, transmucosal absorption (buccal cavity), inhalation of volatilized fentanyl and intravenous injection of contents extracted from transdermal patches. The self-delivery of drug also occurs by simultaneously combining different routes of administration. Herein we describe a case report of two fatal fentanyl poisonings which occurred in a married couple within a five hour time interval. We discuss the available medical histories of the two decedents (including prescribed medications) together with first responder witness reports and significant findings from both autopsies and the postmortem toxicological analyses in each case. Physical evidence included a 75 μg/h fentanyl transdermal patch retrieved from the oral cavity or airway of the female decedent during resuscitative efforts at the scene and a "disrupted" (e.g., cut) 75 μg/h fentanyl transdermal patch recovered in the personal effects of the male decedent at autopsy. The postmortem blood fentanyl concentrations measured in the 45-year-old white female decedent and the 52-year-old white male decedent were 0.050 mg/L and 0.030 mg/L, respectively. Fentanyl was extracted from blood using solid phase extraction and quantitated by SIM GCMS using a previously published procedure.

Key Words: fentanyl, poisoning, blood

CASE REPORT: THE USE OF AMITRIPTYLINE IN DRUG FACILITATED SEXUAL ASSAULT

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Amitriptyline is a tricyclic antidepressant with sedative effects that has been available in the United States since the early 1960's. It is administered in 10-150 mg tablets or as a 10 mg/L injectable solution. While amitriptyline is commonly encountered by the Los Angeles County Department of Coroner Toxicology Laboratory, the circumstances of its use in four Southern California homicides is unique.

Four murders occurred between April 2000 and April 2001. The victims, two females and two males, were initially invited for consensual sex. They were then drugged, sexually abused, bound with ligatures, and ultimately killed. This report reviews four cases of drug-facilitated sexual assault using amitriptyline. The presence of amitriptyline in high concentrations coupled with unique criminalistics evidence aided in the linking of this series of homicides across county jurisdictions.

The analysis of amitriptyline from postmortem specimens consisted of a basic, liquid/liquid (n-butylchloride) extraction procedure with screening and quantitation by GC/NPD. Linearity was achieved from 0.10 to 5.0 mg/L with the limit of quantitation at 0.10 mg/L. Confirmation of amitriptyline was determined using a GC/MS by comparison with an analytical standard.

The following amitriptyline concentrations are reported.

	Amitriptyline (mg/L or mg/kg)				
	Central Blood	Peripheral Blood	Liver	Gastric	Brain
Case #1	—	—	86	19 mg	6.2
Case #2	—	—	76	—	8.3
Case #3	0.90	0.11	1	<0.10 mg	—

Keywords: Postmortem toxicology, drug-facilitated sexual assault, amitriptyline

"THE DOSE MAKES THE POISON", DISCUSSION OF THREE CASES OF LETHAL HEROIN INTOXICATIONS

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Introduction: The lethal dose of heroin differs widely. Tolerance plays a key role. To the persons in the official Swiss heroin program, the drug is often prescribed in high dose. In spite of the medical surveillance, lethal poisoning is not an uncommon finding.

Results: Case A) A 35 y.o. male administered himself (intravenously) under medical observation in 30 min. 400 mg chemically pure heroin and ingested 500 mg Morphine–retard. He then went back home. Two days later he was found death in his flat. Toxicological findings: heart blood: free morphine 1605 ng/mL, M-3-G 686 ng/mL, M-6-G 390 ng/mL. Interpretation: the deceased did not swallow the Morphine-retard capsules. He smuggled them out of the injection place, the so-called "Fixerstübli" and injected them instead. Cause of death: heroin and morphine intoxication. Manner of death: suicide or accident. Case B) A 39 y.o. male came by himself to a hospital with stomach pain. During the medical treatment he received 0.2mg of fentanyl and 5mg diazepam by the medical staff. During his stay he snorted two times a powder which was believed by the nurses to be cocaine. Four hours after the start of the medical treatment he died. Toxicological findings: serum (peripheral) sample taken at the hospitalization: free morphine 267 ng/mL, Codeine 32 ng/mL. Serum from heart blood collected at the autopsy: free morphine 726 ng/mL, codeine 120 ng/mL. Findings: The patient snorted, instead of cocaine, "street heroin". Cause of death: heroin poisoning. Manner of death: suicide or accident. Case C) A 28 y.o. female was found dead in her flat. An injection mark was found in her right antecubital fossa. Toxicological findings: heart blood: free morphine 140 ng/mL, total morphine 693 ng/mL. Results of police investigation: a stranger injected approx. 400 mg of street heroin (purity approx 15%) intravenously on this female on her request. Cause of death: heroin poisoning. Manner of death: killing on request.

Conclusions: High doses of heroin and morphine are needed to satisfy the patients in the official heroin program, and are typically tolerated by them. However, in spite of medical surveillance lethal overdoses are observed. Quite low heroin doses may be lethal for opiate-naïve subjects.

Keywords: heroin, morphine, postmortem

ECSTACY MANUFACTURE: A CASE FOR QUALITY CONTROL

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This case report involves the death of a 25 year old male who was found deceased in his boarding room some 2½ days after last being seen alive. The decedent's body was located face down on his bed. There were no signs of drug use and no medication/illicit drugs were discovered at the scene by attending police.

A full autopsy was performed on the deceased to determine the cause of death. Macroscopic findings indicated putrefactive changes with extensive autolysis of internal organs in keeping with early stages of decomposition. There was marked pulmonary oedema and bilateral hydronephrosis. There were no other significant pathological findings. Specimens submitted for toxicological analysis revealed a free morphine concentration (cavity blood)~60 mg/L; 4.2 mg/kg (liver); total morphine 76 mg/L (urine); and 190 mg of morphine in stomach contents. No 6-acetyl morphine was detected in blood or urine, and no other drugs or alcohol were detected. The cause of death was given as morphine toxicity.

Additional information provided by the family indicated a family history of sleep apnoea. Tablets, located in the decedent's bedroom and which according to the girlfriend belonged to the deceased, were submitted to the laboratory for analysis some weeks after the death. The 3 tablets (green, scored with the markings CK on either side) and initially purchased presumably as ecstacy, were identified as containing 100 mg of morphine with trace amounts of methamphetamine. The decedent had consumed at least two of these tablets or more resulting in his death. This case illustrates the danger of experimenting with illicit drugs and serves as a reminder that information germane to medicolegal death investigation can come from a variety of sources.

The Coroner found that the death was an accidental overdose.

Keywords: ecstacy, morphine, death.

A RETROSPECTIVE STUDY OF OXYCODONE DEATHS IN ONTARIO 1999-2003

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This report presents the results of a retrospective study aimed at determining the role of oxycodone in deaths investigated by the Office of the Chief Coroner in Ontario, Canada, between 1999 and 2003. The objectives of the study were 1) to assess changes over this period in the prevalence of oxycodone in deaths investigated by the coroners, and 2) to assess the extent to which these deaths were oxycodone-induced or oxycodone-related. The cases included in this study were death investigations in which specimens obtained from a medico-legal autopsy, were submitted to the Centre of Forensic Sciences for a toxicological examination. In all but three cases, examinations consisted of analysis for alcohols, a general drug screening procedure that detects a range of therapeutic agents and illicit substances that exhibit central nervous system toxicity and are encountered in postmortem toxicology, and targeted analyses for the quantitation and confirmation of screen findings. Cases were included in this study only when a blood oxycodone finding was available. In the majority of cases, analysis was performed on heart blood, and in others, on samples identified as femoral or peripheral venous, or from an unspecified source. Quantitation of total oxycodone in whole blood was carried out using a liquid/liquid basic extraction, followed by a gas chromatographic analysis using a nitrogen/phosphorous detector and capillary fused silica columns.

A total of 291 cases met the inclusion criteria for this study, representing 194 males and 97 females. Oxycodone-positive cases submitted to this laboratory in 1999 numbered 22 and increased steadily each year to reach 101 in 2003. The increase in annual submissions was markedly disproportionate to the overall increase in submissions. Although no attempt was made in this phase of the study to determine whether the detected oxycodone was due to the ingestion of Oxycontin® or any other oxycodone-containing formulation, it is noteworthy that the increase in the prevalence of oxycodone findings in death investigations coincides with the introduction of Oxycontin® to the Canadian market in about 1996. In order to assess the involvement of oxycodone in these deaths, the coroner's conclusion regarding the cause of death was recorded. In 39 cases over this five-year period, death was attributed to natural causes or trauma, but not related to the presence of oxycodone. Blood oxycodone concentrations ranged from less than 0.01 to 0.9 mg/L.

In the remaining cases (n = 252), death was determined by the coroner to be drug-induced or drug-related. Based on the reported postmortem blood toxicology findings, cases were divided into four categories: 1) oxycodone alone (2%); 2) oxycodone and other drugs in therapeutic concentrations, with ethanol present at or below 50 mg/100 mL (20%); 3) oxycodone and other drugs in potentially toxic concentrations, with ethanol in excess of 50 mg/100 mL (59%); and 4) oxycodone and other drugs in potentially fatal concentrations (19%). The blood oxycodone concentration ranges for each group were 0.1 to 0.92 mg/L, 0.1 to 5.5 mg/L, less than 0.01 to 9.8 mg/L, and 0.03 to 4.4 mg/L, respectively.

The observed overlap in blood oxycodone concentrations across these groups underscores the importance of other factors such as polydrug use, drug formulation, and the experience of the user in assessing the contribution of oxycodone to the death.

Keywords: oxycodone, postmortem, toxicology

ANALYSIS OF POSTMORTEM BONE/BONE MARROW SPECIMENS FOR DRUGS OF IMPORTANCE IN FORENSIC TOXICOLOGY

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To date, there is a paucity of literature published on the determination of drugs in bone and bone marrow. This type of research is important forensically because the extraction of bone specimens may be useful when traditional postmortem samples are not available for toxicological analysis. Postmortem bone samples, taken from the iliac crest in adults and vertebrae in infants, were extracted and analyzed for the presence of benzodiazepines and cocaine and metabolites. The purpose of this study was to correlate the concentration of drugs found in postmortem blood with the concentrations in bone/bone marrow samples. The specimens were thoroughly cleaned by removing all traces of skeletal muscle and tissue and then rinsing with deionized water until the wash ran clear. Two grams of each bone/bone marrow sample was cut into slivers and soaked in 4 milliliters of methanol for sixteen hours (± 0.5 hours). Cocaine and metabolites were extracted from the methanol by liquid/liquid extraction followed by solid phase extraction (SPE), derivatization with MSTFA, and analysis by GC/MS in single ion monitoring (SIM) mode. Benzodiazepines (BDP) were isolated from the methanol extract by liquid/liquid extraction and analyzed by GC/ECD.

The concentration of drugs found in the bone/bone marrow samples was compared to postmortem blood toxicology results. Three of seven cases analyzed for cocaine and metabolites were positive. In one case, cocaine was quantitated at 120 ng/g bone, cocaethylene 80 ng/g bone, and ecgonine methyl ester (EME) was qualitatively positive. The heart blood results in this case were benzoylecgonine (BE) 332 ng/ml, cocaine 144 ng/ml, cocaethylene 32 ng/ml, and EME qualitatively positive. In another case, BE was quantitated at 666 ng/g bone and in the heart blood BE was 522 ng/ml. The third case had 48 ng/g bone cocaethylene and EME was qualitatively positive. The heart blood in this case contained 211 ng/ml BE, 29 ng/ml cocaine, and EME was qualitatively positive. The remaining four cases were negative for cocaine and metabolites in the bone/bone marrow and the blood concentrations for BE and cocaine ranged from 123 ng/ml-394 ng/ml and 24 ng/ml-29 ng/ml respectively. These positive cases demonstrate that cocaine and metabolites can be detected in bone, but the presence of specific analytes may differ from those present in the blood and the concentration of the analytes in bone/bone marrow may be higher or lower than that in the blood. Therefore, these results should be interpreted cautiously.

Six cases were examined for the presence of BDP. Of these six cases, four were positive for diazepam with values ranging from 0.60 ug/g bone-2.4 ug/g bone, four were positive for desmethyldiazepam (DMD) with values ranging from 0.32 ug/g bone-1.6 ug/g bone, and one case was unsuitable for analysis. In the cases where diazepam was positive in the bone/bone marrow, the heart blood concentrations ranged from 0.13 mg/L-0.39 mg/L. DMD in the heart blood ranged from 0.10 mg/L-0.67 mg/L. In every case where diazepam and/or DMD were present in the heart blood, both were also recovered in the bone/bone marrow.

These results demonstrate that bone/bone marrow specimens may be utilized as an alternative matrix for postmortem toxicological analysis where traditional matrices are not available. The preliminary data on cocaine and metabolites and BDP failed to show a linear correlation between bone/bone marrow and blood drug concentrations. It appears that the likelihood of detecting a drug in bone/bone marrow specimens depends on the type of drug being analyzed. Future investigation of other drug classes such as opiates, basic, and acidic/neutral drugs may provide additional information for the elucidation of correlation data between bone/bone marrow and blood specimens.

KEYWORDS: Bone, Cocaine, Benzodiazepine

DRUG RELATED DEATHS IN THE CITY AND COUNTY SAN FRANCISCO

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Aims: The timely detection of drug-related health problems and emerging drug trends depend, at least in part, on information provided by coroners and medical examiners. To assist in this process we reviewed all drug-related deaths occurring in our jurisdiction during 2002.

Design: Retrospective analysis of all death investigations carried out by the San Francisco Office of the Chief Medical Examiner (SFOCME), from January 1, 2002 until December 31, 2002, where postmortem toxicology revealed the presence of psychoactive or abused drugs, and complete autopsy performed.

Methods: If available, urine is first screened with EMIT testing and results confirmed by GC/MS or LC/MS. In the absence of urine, GC/MS is used to screen blood, liver, CSF or other biological specimens directly.

Findings: The SFOCME serves a population of approximately 750,000, and this number has been stable for several decades. In 2002, 1463 cases came under the jurisdiction of our office, and drugs were detected in 257 of these cases. Cocaine or benzoylecgonine was found in the blood and/or urine of 153 (59%) cases. Morphine and/or codeine were detected in the blood or urine of 119 (46%), and methamphetamine was present in 19% (48/257). Poly-drug abuse was common among the cases which came under our jurisdiction. Morphine was present in nearly a third (72/257, 28%), of the cocaine users. The occurrence rate for other drugs was much lower; methadone was present in 33 cases, oxycodone in 23, fentanyl in 7, hydrocodone in 6, MDMA in 5, and PCP in 2 cases.

Conclusions: The number of methamphetamine-related deaths continues to be remarkably stable within our jurisdiction and is, in fact, lower than in 1994, when 52 deaths were recorded (J Forensic Sci. 1999 Mar; 44(2):359-68). The rate for methadone related deaths also appears to be stable, while the number of oxycodone-related deaths appears to be increasing, suggesting that allocating additional resources to treat methadone diversion may be unnecessary in our jurisdiction. On the other hand, the increasing number in fentanyl-related deaths suggests that diversion of Duragesic® patches in our jurisdiction is an increasing problem worthy of further investigation.

Keywords: Drug, Epidemiology, San Francisco

THE ROLE OF COCAINE IN HEROIN RELATED DEATHS. HYPOTHESIS ON THE HEROIN-COCAINE INTERACTION

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In recent years, drugs-of-abuse related deaths involving cocaine observed at the Department of Legal Medicine & Public Health of Pavia have shown an increase, probably reflecting the rising trend in cocaine use in Western Europe: "pure" cocaine deaths have increased from 6 cases in 1979-1991 (1.5% of drug-of-abuse deaths) to 13 in 1992-2002 (3,2%), and in the same periods heroin related deaths (HRDs) involving cocaine amounted to 8 (1.9%) and 22 (5.4%), respectively. In the attempt to investigate the role of cocaine in HRDs, acute narcotism cases testing positive for cocaine and/or metabolites in blood (>10 ng/ml, COC+) were examined. Only cases occurred from 1997 to 2001 were considered as in this period all data were obtained using the same analytical procedures (free morphine and total morphine by DPC Coat-A-Count radioimmunoassay before and after enzymatic hydrolysis, cocaine and metabolites in blood by SPE, TMS derivatization and GC-MS). The median, minimum and maximum concentration of free morphine (FM) and total morphine in blood (TM), urine (UM) and bile (BM) in the COC+ group (n=9) were compared with those calculated in the group of "pure" HRDs (no other drugs detected in blood, COC-, n=30). Differences among the two groups were statistically evaluated using the two-tailed Mann-Whitney U Test. Statistical analysis was also carried out including in both groups cases testing positive (>2 mg/dL) for blood alcohol (COC+, n=19; COC-, n=76). The median TM was found to be lower in the COC+ group (0.32 mg/l *vs.* 0.90 mg/l, P=0.0214) and also the median FM (0.08 mg/l *vs.* 0.28 mg/l, P=0.1064). The FM/TM ratio was similar in the two groups (0.33 and 0.35) and also UM (21.0 mg/l and 18.0 mg/l), whereas BM was higher (90.0 mg/l *vs.* 49.0 mg/l, P=0.0268). Similar results were obtained by repeating statistical analyses after including in the two groups cases with positive blood alcohol concentration (BAC). This picture is very different from what was previously observed for the heroin-ethanol interaction in HRD cases (A. Polettini *et al.*, J. Anal. Toxicol., 23, 570, 1999), and updated with more recent data: in the high-ethanol (HE, BAC >100 mg/dl) group TM was lower than in the low-ethanol (LE, BAC≤100 mg/dl) group (0.59 mg/l *vs.* 0.90 mg/l, P=0.0180), the FM/TM ratio was higher (0.66 *vs.* 0.43, P=0.0038), FM was equal, UM was lower (0.21 mg/l *vs.* 26.5 mg/l, P=0.0001), and so BM (10.0 mg/l *vs.* 26.5 mg/l, P=0.0156). Indeed, the observed lower TM in the COC+ and in the HE groups support the hypothesis of an interaction of both cocaine and ethanol with heroin in the occurrence of death. Ethanol results suggest that a pharmacokinetic interaction is prevalent (inhibition of heroin metabolism, as suggested by the increased FM/TM ratio, resulting in reduced urinary and biliary excretion). In the case of cocaine, a pharmacodynamic interaction seems to prevail, as the FM/TM ratio remains unchanged and UM and BM are not lower in the COC+ group. This hypothesis could not be confirmed owing to the paucity of data and the many uncontrolled variables involved.

Keywords: heroin related death, cocaine, interaction

DRUG EXPOSURE PATTERN IN HAIR AND FEMORAL BLOOD IN DECEASED DRUG ADDICTS

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Blood drug levels of illicit drugs are often not particularly high in postmortem overdose cases. This is true for amphetamine and cocaine as well as for opiates. Thus, the postmortem investigations do not support the use of the word "overdose", since the dose taken in these cases is probably not much larger than is usually taken by chronic addicts. One explanation for this outcome may be reduced drug tolerance following a period of abstinence, but – although some epidemiological reports suggest a recent interruption in drug use in some cases -there are no firm data to confirm this hypothesis. It is difficult to estimate the degree of drug tolerance in deceased subjects, but hair analysis of illicit drugs may at least provide a map of the past and recent drug use pattern. In the present study, segmental hair analysis was applied on hair samples collected from 40 deceased, supposedly drug addicts, including both cases of probable drug overdoses, and cases with other causes of death. Portions of the hair samples were analyzed with an LC-MS-MS screening method, which covers amphetamine and -derivatives, cocaine and metabolites, morphine, 6-monoacetylmorphine (6-MAM), codeine, diazepam, flunitrazepam, nicotine and cotinine. Positive findings, except nicotine and cotinine, were confirmed with GC-MS verification methods. For these analyses, the hair was carefully aligned and cut in short segments (the first three segments 5 mm each, and additional segments 10 mm long). The verification methods also included washing steps, and analysis of washings was carried out to control for external contamination. Femoral blood, urine and vitreous humor were also collected at the postmortem examinations for the routine toxicological drug screen. Twelve of the cases were classified as heroin overdoses by the responsible forensic pathologist. In Table 1 the number of detections of seven different drugs/metabolites in hair and blood (results for benzodiazepines not shown) in these 12 cases are displayed. In most cases, a marked change in abuse pattern was evident. Hence, a gradual decrease in morphine and/or 6-MAM levels was observed in 6 of 12 cases. It was also found that the drug use at time of death, as reflected by the drugs detected in femoral blood, more often than not differed from the drug use pattern seen in hair. Amphetamine, cocaine, benzoylecgonine and 6-MAM were more often detected in hair than in blood. The opposite was true for codeine and morphine. In four cases, fentanyl was found in femoral blood in concentrations that suggest that this drug most likely was the main or a contributory factor for the demise. The results from the segmental hair analyses showed that the final heroin dose in four cases obviously had been preceded by a period of lowered drug use, or abstinence, whereas in other cases, a continuous drug use before death was disclosed. Thus, reduced tolerance may be of importance in certain cases, but in the majority of the cases, other factors seem to be more important. Interestingly, in at least four of these twelve "overdose" cases, there was evidence of a delayed death, leading to a drop in blood morphine levels. This observation underscores the importance of considering autopsy findings and circumstantial information when interpreting postmortem toxicological data - the finding of low morphine levels, and/or lack of 6-MAM does not exclude an acute opiate intoxication. It is concluded from the present study that hair analysis can assist in the toxicological evaluation of postmortem cases, and that segmental analysis can improve the interpretation further.

Table 1. Number of detections of different drugs of abuse in hair and blood from 12 deceased heroin addicts classified as overdoses.

	Codeine	Morphine	6-MAM	Amphetamine	Cocaine	Benzoylecgonine	Fentanyl
Hair	5	5	9	5	2	2	n.a.
Blood	7	12	3	3	0	1	4

Key words: Hair analysis, opiates, substance abuse

P31

TRENDS IN THE DETECTION OF DRUGS OF ABUSE IN POSTMORTEM SAMPLES

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Introduction: Drug misuse is a major social and economic problem facing the Grampian and Highland regions of Northern Scotland. The Oil and Fishing industries have brought wealth and reduced unemployment in these regions. Consequently, drug dealers have targeted these areas as a potential market for the selling of drugs. As a result, there has been a dramatic increase over the last decade in the number of samples screened for drugs-of-abuse in Grampian. Unfortunately, the rise in drug misuse has also seen an increase in the number of drug related deaths in Grampian and Highland over this time. Fatalities from suspicious causes are frequently associated with illegal drug misuse. We have assessed the trends of the presence of commonly abused illicit drugs in post-mortem specimens from individuals who died under suspicious and accidental circumstances in Grampian and Highland from 1994 to 2000.

Methods: Post-mortem data was obtained from an archival laboratory database of forensic cases for accidental or suspicious death over the period 1994 to 2000. Drug trends were assessed in cases in which at least one drug was detected. The drugs chosen for this investigation included Amphetamine, 3,4-Methylenedioxyamphetamine (MDA), 3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy), Cannabinoids, Benzoylecgonine, Cocaine, Codeine, Diazepam, Dihydrocodeine, Methadone, Morphine and 6-Monoacetylmorphine, since they represented the most commonly abused drugs found in Grampian and Highland. Drug detection was assessed from post-mortem blood and urine specimens collected into plain tubes and extracted by liquid-liquid or solid-phase extraction using either GC-MS or HPLC for detection.

Results: A total of 2993 forensic cases were referred for analysis over the six year period. There was no evidence that the number of cases was increasing (mean = 458 cases per year, regression coefficient = 9.57, $p = 0.51$). From these cases, 641 positive drug screens were obtained from a total of 330 individuals. A rising trend (regression coefficient; significance level) from 1994 to 1999 was observed for the presence of Cannabinoids (6.29; 0.002), Codeine (5.77; 0.006), Diazepam (6.89; 0.002), Dihydrocodeine (3.26; 0.002) and Morphine (7.43; 0.004). Overall, a rising trend was observed for the presence of at least one drug (regression coefficient 13.89; $p = 0.001$), with the finding of more than one drug being present also increasing.

Conclusions: From 1994 to 2000 the number of positive drug tests in post-mortem specimens from this source are increasing on average by 37 cases per year. Of these, the number of patients with more than one positive drug is also increasing. This would fit in with the view that drug abuse is increasing within society, as is multiple drug use. Overall this study provides evidence that drugs of abuse are increasingly implicated or associated with death from suspicious causes. This evidence of the increasing involvement of drug abuse in fatalities arising from suspicious and accidental causes will strengthen the demand for increasing specialised toxicological analysis by the Procurator Fiscal, which will have profound economic and resource implications upon laboratories both in Grampian and nationally.

METHOD FOR CARBON MONOXIDE DETERMINATION IN BLOOD USING GAS CHROMATOGRAPHY WITH METHANE AS AN INTERNAL STANDARD

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A gas chromatographic procedure for determination of carboxyhaemoglobin in blood with a thermal conductivity detector is commonly used. Processing of blood samples for the measurement of liberated carbon monoxide and obtaining reproducible results, especially in cases of postmortem blood samples, is performed using a method with methane as an internal standard. An apparatus for postmortem blood sample preparation for CO liberation and subsequent GC determination is presented together with a description of the method that includes schematic drawings. A core part of the device is a simple reaction tube partly filled with saturated NaCl solution. Blood, diluted 1:1 with distilled water, is added into the tube. Then the tube is closed and deaerated through a rubber septum. Through the same septum, a ferricyanide reagent and a gas mixture of H_2 and CH_4 (10:1) are added. The addition of reagents and gas phase is performed in a system of communicating vessels. Methane serves as an internal standard. The reaction mixture is shaken and an aliquot part of the gas phase is injected into the gas chromatograph (chromatographic column packed with molecular sieve 5A, isothermal conditions 80°C , TCD, carrier gas: hydrogen). A blood sample saturated with carbon monoxide up to 100% is processed in the same way. Total concentration of bonded CO in the sample is calculated from peak areas of analysed sample and 100 % saturated one.

$$\%CO = \left(\frac{A_{CO}}{A_{CH4}}\right)_{sample} \times \left(\frac{A_{CH4}}{A_{CO}}\right)_{100\%} \times 100\ \%$$

The calibration curve was obtained on the basis of measurements of intermixture fully saturated blood sample and negative one in required ratios.

The method was validated with following parameters:
COHb calibration in the range of 0 - 100%

$y = 0{,}0031x + 0{,}0045$
$R^2 = 0{,}9973$
LOD = 0,44 % COHb
LOQ = 1,47 % COHb

The method is applicable even in cases of decomposed blood. Replicate injection of the homogenous gas phase into the gas chromatograph may be performed. The precision of the determination expressed as coefficient of variation deteriorates in cases of lower concentration of COHb.

Keywords: blood sample preparation, carbon monoxide, internal standard

POSTMORTEM CASES RELATED TO COCAINE IN THE NETHERLANDS

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Cocaine is a popular drug and is often encountered in postmortem toxicology cases. In this study, we present cocaine-related deaths in the Netherlands from October 2002 to October 2003. In this period, the presence of cocaine, benzoylecgonine and methylecgonine was demonstrated in 45 cases in which cocaine concentrations were 0.02 mg/l or higher. Of these 45 cases, 34 were male. The 45 deaths were divided into the following categories:

1. anatomical cause of death, cocaine present with or without ethanol and other drugs (ACD, n=17)
2. toxicological cause of death, cocaine as most likely cause (TCD, n=12)
3. toxicological cause of death, cocaine and other drugs present, other drug(s) as most likely cause or combination (TCD+, n=6)
4. no clear cause of death, cocaine present with or without ethanol and other drugs (NCD, n=8)

In 12 of these 45 cases, cocaine-overdose (TCD) was the most probable cause of death. In these 12 cases, mean concentrations (± standard deviation) of cocaine, benzoylecgonine and methylecgonine in femoral blood were 8.0 (±4.9) mg/l, 9.4 (±4.0) mg/l and 7.0 (±3.9) mg/l, respectively. In eleven of these cases, packages were found in the stomach. The other case was most probably a fatal case of cocaine delirium with a cocaine concentration in femoral blood of 0.23 mg/l, a benzoylecgonine concentration of 7.0 mg/l and a methylecgonine concentration of 1.64 mg/l.

The mean concentrations of cocaine and metabolites in femoral blood in the TCD+ category were much lower compared to the TCD category: the concentrations of cocaine, benzoylecgonine and methylecgonine in these cases were 0.54 mg/l, 1.99 mg/l and 1.21 mg/l, respectively. In these cases of TCD+, combinations with ethanol, amphetamines, benzodiazepines, methadone, morphine and GHB were found.

Compared to the TCD and TCD+ category, the mean concentrations of cocaine and metabolites in femoral blood in the categories ACD and NCD were much lower: the mean concentrations were 0.10 mg/l and 0.18 mg/l for cocaine, 1.11 mg/l and 1.91 mg/l for benzoylecgonine and 0.25 mg/l and 0.45 mg/l for methylecgonine , respectively. In the cases of anatomical cause of death (ACD), there were two cases in which cocaine was the only drug present. In the other 15 cases of ACD, combinations of cocaine with ethanol and other drugs (amphetamines, benzodiazepines, methadone and morphine) were found. Out of 8 cases where no clear cause of death (NCD) was found, 7 cases had combinations of cocaine and ethanol or other drugs (benzodiazepines, methadone and morphine). There were no striking differences between the categories TCD+, ACD and NCD in combinations of drugs found together with cocaine.

In two of the total 45 cases, the cause of death could be anatomical as well as toxicological. One of these cases was a woman with packages in the stomach and one package in the trachea (mechanical obstruction): the concentrations of cocaine, benzoylecgonine and methylecgonine in femoral blood were 3 mg/l, 4.3 mg/l and 4.7 mg/l respectively. The other case was a shooting; a cocaine concentration in femoral blood of 1.9 mg/l was found.

Keywords: postmortem blood concentrations, cause of death, cocaine

DETERMINATION OF PERMETHRIN, ETHYLBENZENE, XYLENE AND THEIR METABOLITES IN AN ACUTE INGESTION OF INSECTICIDE

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The case history and toxicological findings of an acute ingestion of the insecticide permethrin in a 7 year old female found in bed with vomit and blood on her face are presented. Permethrin and its carriers, ethylbenzene and xylene, are all components found in the commercial product, Atroban. The concentration of permethrin was measured in the liver, aortic blood and gastric content using gas chromatography-mass spectrometry (GC/MS). Concentrations of ethylbenzene and xylene were measured in the same biological samples by headspace-gas chromatography. Blood and liver concentrations of permethrin, ethylbenzene and xylene were detected at concentrations below the lowest curve calibrator. Therefore, permethrin and ethylbenzene were reported as < 1.0 mg/L(kg), and xylene as <3.0 mg/L(kg) in the blood and liver samples. Permethrin, ethylbenzene and xylene concentrations in the gastric content were 5400 mg/kg, 2900 mg/kg, and 13000 mg/kg, respectively. Additionally, the contents of an unlabeled bottle found at the scene were collected and sent to the Medical Examiner's Office for evaluation. The following concentrations of permethrin, ethylbenzene and xylene in this sample were 6400 mg/L, 3200 mg/L, and 16800 mg/L, respectively. The trimethylsilyl derivative of a urine extract was also prepared. GC/MS analysis of the urine extract allowed for the identification of the metabolites of xylene (*o*-, *m*-, and *p*-methyhippuric acid, and *o*-, *m*-, and *p*-toluic acid), ethylbenzene (mandelic acid and phenylglyoxylic acid), and the normal urinary organic acids of a child (urea, hippuric acid and citric acid). The relative concentration of the contents of the unlabeled bottle and the gastric content were consistent with ingestion of the contents of the bottle. The cause of death was ruled as insecticide poisoning - aspiration of the organic solvents in the insecticide.

Keywords: Permethrin, Insecticide poisoning, Postmortem

CARBON MONOXIDE AND ETHANOL IN FIVE VICTIMS OF A FIRE FATALITY

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Several reports have been published about carbon monoxide and ethanol in fire fatalities, but possible physiological interaction between these toxic agents is still under discussion. We report a tragic mattress fire that occurred in a small room at a prison where five young men under the influence of alcohol died. Blood specimens were obtained from heart of victims and tested for ethanol by headspace gas chromatography (HS-GC/FID) . Shimadzu GC-14, CR 4A with FID detector, stainless steel column packed with 0.3% carbowax 1500 graphapac 60/80 and tert butanol internal standard were used . Percent of carboxihemoglobin (%COHb) was determined by microdifussion methods. Additionaly, micro quantitative determination of cyanide was determined according to the method proposed by Gelttler and Goldbaum. Comprehensive testing for drugs of abuse, psychotopic drugs and other therapeutic drugs was performed by HPTLC. The %COHb and ethanol (g/L) in the five victims were: 60% and 0.54 ; 35 % and 3.99; 55% and 0.39; 45% and 0.70, 50% and 0.81 respectively. No other components with toxic relevance were found. According to this study we concluded that there was no evidence for an ethanol protective effect against carbon monoxide poisoning.

Keywords: carbon monoxide, ethanol, fire

METHAMPHETAMINE IN HAIR AND INTERPRETATION OF FORENSIC FINDINGS IN A FATAL CASE

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Hair analyses for drugs are considered to be a significant tool for distinguishing between recent and long term drug abuse in forensic and clinical toxicology. Chronic consumption of drugs can gradually induce certain harmful effects on the human organism and it can exacerbate some pre-existing diseases. Neither analyses for drugs in blood or urine in isolation can yield sufficient information about the history of a person and their results can not be correlated directly with the toxic effects displayed. The chronic abuse of methamphetamine is known to be connected with cardiovascular diseases. During or after autopsy certain types of morphologic alterations are found in the hearts of stimulant addicts. The rapid increase of blood pressure after a methamphetamine intravenous dose can be risky for addicts with arteriosclerosis. However, the anamnestic data about a deceased person may not always be available to explain the pathological findings and to clasify the cause of death correctly.

The aim of this presentation is to demonstrate the usefulness of hair analyses for drugs in the context of explaining the development of pathological cardiovascular alterations found after the autopsy of a case where methamphetamine consumption was involved. In this case (31 year old man) only methamhetamine and metabolites were detected with traces of ephedrine which is the precursor in illicit synthesis of Pervitin. The femoral blood level of methamphetamine was 1500 ng/ml. It was documented by a witness that the man died within one hour after an intravenous injection of the drug. The cause of death was established as cerebral edema due to cerebellar bleeding shortly after an intravenous dose of methamphetamine. Findings of methamphetamine in the first three 2 cm hair segments numbered from the roots were nearly equal (132 ng/mg), in the fourth 2 cm segment it was approximately a half of previous values. In the remaining, distal 7 cm hair segment sample the value of methamphetamine was comparable to the first one. These results provide clear evidence that the man had been a chronic methamphetamine abuser for about more than a year and this information can help to explain the pathology, the consequence of which could be the bleeding into the cerebellum after the last single methamphetamine dose.

Keywords: forensic toxicology, amphetamines in hair, methamphetamine chronic toxicity

QUANTITATIVE APPROACH TO DRUG ALCOHOL INTERACTION BASED ON POSTMORTEM DATA

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Our objective was to investigate the drug-alcohol interaction in terms of postmortem concentrations of both drugs and alcohol in fatal poisonings. As our target analytes, we chose the three drugs most commonly causing fatal single-drug poisonings in Finland: the tricyclic antidepressant amitriptyline, the opioid analgesic propoxyphene and the phenothiazine antipsychotic promazine. The effect of benzodiazepines (BDZs) on drug and alcohol concentrations was also evaluated in the study.

The laboratory database was searched for fatal poisonings caused by A) amitriptyline, propoxyphene or promazine alone, B) one of these drugs with alcohol present, or C) alcohol (ethanol) with one of these drugs detected. In order to increase sample size, cases with therapeutic or subtherapeutic concentrations of some of the most frequently occurring BDZs were also included, but separate statistical treatments were performed to evaluate their effect. The drug and alcohol concentrations included in the study had been measured in femoral venous blood. The final data set consisted of 116 amitriptyline cases, of which 60 (52%) BDZ positive, 120 propoxyphene cases, of which 43 (36%) BDZ positive, and 96 promazine cases, of which 42 (44%) were BDZ positive.

The blood drug concentrations found in the unmixed amitriptyline poisonings were compared with those detected in the amitriptyline-alcohol cases, and likewise for propoxyphene and promazine. The blood alcohol concentrations found in the drug-alcohol poisonings were in turn compared with those in a previously compiled reference group consisting of fatal alcohol poisonings caused by alcohol alone (n=615).

The median drug concentrations in unmixed drug poisonings (BDZ+) and drug-alcohol poisonings (BDZ+) were 3.5 (2.1) mg/l and 1.9 (0.75) mg/l for amitriptyline, 8.2 (4.9) mg/l and 6.1 (2.7) mg/l for propoxyphene, and 6.3 (7.6) mg/l and 6.7 (5.1) mg/l for promazine. Regarding the effect of BDZs, the difference between the median drug concentrations was statistically significant in amitriptyline poisonings with and without BDZs ($p<0.05$); amitriptyline-alcohol poisonings with and without BDZs ($p<0.001$); and propoxyphene-alcohol poisonings with and without BDZs ($p<0.01$). However, comparing unmixed drug poisonings with corresponding drug-alcohol poisonings, there were no significant differences between the median drug concentrations.

The median alcohol concentrations in drug-alcohol poisonings (BDZ+) were 1.7 (1.9) ‰ in amitriptyline-alcohol poisonings, 1.6 (1.5) ‰ in propoxyphene poisonings, and 1.0 (1.4) ‰ in promazine poisonings, while in alcohol poisonings by alcohol alone, it was 3.3 ‰, differing significantly from all of the medians above. The presence of BDZs did not show a significant effect in these comparisons.

We also divided the drug-alcohol poisonings into four (per each drug) roughly equal sized bins, according to the drug concentration detected, with the lowest concentration group averaging ~ 2-3 times the upper limit of the corresponding therapeutic range. Interestingly, already in the lowest concentration groups, the distribution of blood alcohol concentrations was very different from that seen in pure alcohol poisonings, and going towards higher concentrations, there was not much further change. In other words, blood alcohol concentrations were similar whether the drug overdose was small or large.

Therefore, we conclude that in the interpretation of forensic toxicology results, even a moderate concentration of alcohol should be considered seriously in the presence of amitriptyline, propoxyphene or promazine, even when their concentrations only slightly exceed the therapeutic range.

Keywords: drugs, alcohol, postmortem

TREND OF ACUTE NARCOTISM DURING THE LAST TEN YEARS

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Since 1993 the Laboratory of Forensic Toxicology at the Catholic University of Rome has evaluated the trends of deaths from narcotism. .We have already studied previous periods and our data were compared with D.C.S.A. (Dipartimento Centrale Servizi Antidroga - Central Antidrug Department) and GTF (Gruppo Tossicologi Forensi - Italian Forensic Toxicologist Association) informations. In this paper we present a statistical analysis of the lethal intoxications in the period 2000-2003 and compare to two prior periods (period A 1993-1996; period B 1997-1999).

Annually cruel deaths observed by the Legal Medicine Institute of Catholic University range between 300 to 400. About 18-22% of these cases are submitted for toxicologic analyses; less than 10% of total deaths could be referred to lethal intoxications.

Analysis of data showed interesting observations relative to the last ten years:

- A decreasing in overdose cases
- Morphine alone is still the main drug accounting for death
- An increase in methadone-related-deaths
- A slight reduction in cases of acute cocaine intoxications

A separate study on morphine and ethanol related deaths was performed; this study showed that the presence of ethanol higher than 0.5 g/l was more significant when morphine concentrations ranged between 20 and 250 ng/ml.

Our study suggests the usefulness of periodic review of mortality phenomena related to drug intoxications.

Keywords: acute narcotism, statistical evaluation, postmortem

FORMALIN-INDUCED METHAMPHETAMINE DECOMPOSITION IN HUMAN LIVER

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Methamphetamine is methylated in the presence of aqueous formalin mixtures within hours at room temperature. The product, N,N-dimethylamphetamine, is also found in human liver exposed to methamphetamine followed by incubation with formalin. In the present study, we determined if liver samples from confirmed methamphetamine abuse subjects would produce N,N-dimethylamphetamine upon formalin fixation. Human liver samples were obtained from four deaths that were investigated by the West Virginia Office of the Chief Medical Examiner. Full toxicological analysis was conducted on samples from the decedents and methamphetamine was among the positive findings in each case. The method used to expose the tissue to formaldehyde involved treating a small piece of liver (100-200 mg) from each case with formalin solution (20% v/v) for 24 hrs at room temperature. The formalin treated tissues were homogenized and the resulting suspension was sonicated for 5 min and then centrifuged. Supernatant aliquots (10 µl) were added to 500 µl of 0.1% formic acid in acetonitrile for mass spectrometric analysis on a Finnigan LCQ DECA ion trap mass spectrometer with an electrospray ionization source. Positive ion spectra recorded in MS, MS^2 and MS^3 modes were used to confirm the presence of N,N-dimethylamphetamine in the mixture, as well as the absence of methamphetamine. Liver tissue not treated with formalin did not contain a detectable level of N,N-dimethylamphetamine. The results suggest that embalmed tissues may give false negative findings for methamphetamine. Detection of N,N-dimethylamphetamine in embalmed liver is a potential surrogate indicator of methamphetamine ingestion.
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Key words: Formalin-induced, Methamphetamine, Methylation

FATAL METHADONE INTOXICATION IN AN INFANT

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We present the case history and toxicological findings of an infant fatality involving methadone. A 10-month old infant was found unresponsive in a crib by her mother. The infant was taken to hospital; however, she was cold and stiff on arrival and was pronounced dead. Few details regarding the case history were known at the time, and the autopsy findings were unremarkable. Specimens were submitted for a full toxicological analysis, including an alcohol analysis by headspace gas chromatography with flame ionization detection; a screen for drugs of abuse and several prescription drug classes using an enzyme-linked immunosorbent assay technique (ELISA); and a screen for basic compounds using gas chromatography-mass spectrometry (GC-MS). Positive findings were confirmed and quantitated using GC-MS. Methadone was detected in subclavian blood at a concentration of 0.67 mg/L. This unexpected result initiated a police investigation, and it was revealed that the mother had fed methadone to her infant in the baby's formula for several months to stop the infant from crying. The mother was on a methadone maintenance program and was given take-home doses of liquid methadone. On the day of the infant's death, the mother admitted to administering twice the amount of methadone she normally gave her child. The cause of death was determined to be "*methadone intoxication*", and the manner of death was "*homicide*". A discussion of the case circumstances, the toxicology findings and methadone pharmacokinetics will be presented.

Keywords: Methadone, Infant, Intoxication

SURVEY OF ABUSED DRUGS IN PERIOD 1986-2003 IN WEST BOHEMIA (MIDDLE EUROPE)

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West Bohemia's area is about 11 000 km^2 and has a population of 850 000 people. The center of the region is Pilsen. After the fall of Communistic regime in 1989, the number and spectrum of drug abuse cases has been gradually increasing. Before 1989, abuse of volatile substances (like toluene) and opiates (especially codeine) was prevalent. After 1989, the production and abuse of methamphetamine (pervitin) has significantly increased. The amphetamine derivatives are the most frequent abused drugs in this region as well as in the whole country. The number of cases has increased seven fold in the last decade to a total of 620 cases.. Most of deaths connected with drug abuse and overdosage are caused by methamphetamine. Occurrence of opiates (mostly heroin) and cocaine is less common – often in Asiatic communities (202 cases of opiates and 12 of cocaine). In the last two years, occurrence of cannabinoids has also increased; reports have doubled from 2001 to 2003 to a 388 cases. The overall increase in drug abuse has also caused an increase in drugged drivers. From statistics it follows that the most dangerous drug in traffic are cannabinoids. Four fatal road accidents caused by drivers under the influence of THC were reported in the year 2003 (three drivers and three other road users died). There was one fatal road accident caused by methamphetamine (the driver and his front passenger died) in 2003).The first and so far unique case of lethal paramethoxyamphetamine (PMA) intoxication in the Czech Republic was recorded in our laboratory in 2001. It was a 21 year old man who had a blood PMA concentration of 1.3 mg/l. Included in these cases are two veterinary surgeons (39 and 41 years) who died in connection with ketamine abuse. The first one had been abusing this substance for a long time and shot himself. The other one committed suicide with combined ketamine, succinylcholine and alcohol intoxication.

Keywords: paramethoxyamphetamine, methamphetamine, cannabinoids

ENANTIOMERIC ANALYSIS OF EPHEDRINES AND NOREPHEDRINES

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Concerned with variations in abuse potential and control status among various isomers of ephedrines and norephedrines, this study was conducted to develop an effective method for the simultaneous analysis of nine structurally related ephedrine-type compounds. Select cold medications were then analyzed to characterize the enantiomeric compositions of ephedrine, norephedrine (phenylpropanolamine or PPA), pseudoephedrine, norpseudoephedrine (cathine), and cathinone.

Among various approaches investigated, a 60-m HP-5MS (0.25 mm ID, 0.25 μm film thickness) was found to successfully resolve the following compounds of interest that were derivatized with (–)-α-methoxy-α-trifloromethylphenylacetic acid (MTPA): (+)-cathine, (+)-cathinone, (–)-cathinone, (+)-ephedrine, (–)-ephedrine, (+)-PPA, (–)-PPA, (+)-pseudoephedrine, (–)-pseudoephedrine. A (–)-cathine standard was not available, but should also be resolvable using this analytical procedure. The injector temperature was set at 250°C and the oven profile was 160-220°C at 5°C/min, hold 1 min, 220-250°C at 25°C/min. This method was applied to the analysis of various over-the-counter cold medications and the results derived from three samples are shown in Table 1. These compounds were isolated from the cold medications utilizing a simple liquid/liquid extraction with ethyl acetate. This method has proven to be an efficient procedure for the separation and identification of various enantiomeric ephedrine and norephedrine-type compounds.

Table 1. Enantiomeric composition (μg/mL) of various ephedrines and norephedrines found in various cold remedies

Sample	(+)-Cathinone	(+)-PPA	(–)-Cathinone	(–)-PPA	(+)-Cathine	(+)-Ephedrine	(–)-Ephedrine	(–)-Pseudoephedrine	(+)-Pseudoephedrine
2	—	—	—	—	—	—	1.84	—	0.565
16	—	—	—	—	—	0.549	0.414	—	48.1
19	1.35	1.19	26.8	2130	340	—	—	—	—

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Key Words: Ephedrine, Norephedrine, Enantiomer

"ECSTASY" IN THE A.M. AND P.M. – MDMA CONCENTRATIONS IN FATALITIES FOLLOWING HOSPITAL ADMISSION

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Over the last 15 years numerous deaths involving "Ecstasy" (MDMA) have been reported and described in the literature. With most cases, either ante-mortem (AM) or post-mortem (PM) concentration data are available. Due to the wide range of results and potential idiosyncratic nature of MDMA toxicity, interpretation of either data set is difficult. The possible influence of post-mortem redistribution may also be a factor, however it has been suggested that most amphetamine analogues are less influenced by this phenomenon than some other drugs. The aim of this study was to evaluate the concentrations of MDMA found in 5 fatalities admitted to hospital where both ante-mortem and post-mortem blood samples were available. Concentration ratios between PM and AM samples were also calculated to compare case data and evaluate redistribution.

Case 1: 31 year old male admitted to hospital following suspected injection of crushed amphetamine and "Ecstasy" tablets. He developed malignant hyperpyrexia and later died. An AM serum MDMA concentration of 1.22 mg/L was measured (45 minutes prior to death) with a corresponding PM (brachial) blood concentration of 2.37 mg/L. This produced a PM:AM concentration ratio of 1.9. Amphetamine and ethanol were also detected.

Case 2: 30 year old male recovered from a river after a night out drinking. He was admitted to hospital but later died. 6 AM samples were analysed (between 9-20 hours prior to death). An AM plasma MDMA concentration of 0.31 mg/L was measured (9 hours before death) with a PM (trunk) blood concentration of 0.47 mg/L (PM:AM ratio 1.5) and PM (arm) concentration of 0.52 mg/L (PM:AM ratio 1.7). Chlordiazepoxide and ethanol were also detected.

Case 3: 26 year old male found collapsed in the street after having taken several "Ecstasy" tablets. He was admitted to hospital but later died after suffering hyperpyrexia. An AM blood MDMA concentration of 2.04 mg/L was measured (1 hour prior to death) with a corresponding PM (femoral) blood concentration of 2.25 mg/L) and PM (jugular) blood concentration of 2.99 mg/L. These produced PM:AM concentration ratios of 1.1 and 1.5, respectively. A trace of paracetamol was also detected but no ethanol.

Case 4: 22 year old female admitted to hospital with hyperthermia following ingestion of approximately 12 "Ecstasy" tablets. An AM serum MDMA concentration of 4.33 mg/L was measured (1 day prior to death) with corresponding PM blood concentrations of 7.25 mg/L (left femoral), 6.19 mg/L (right femoral), 28.39 mg/L (heart) and 11.93 mg/L (vitreous humor). These produced PM:AM concentration ratios of 1.7, 1.4, 6.6 and 2.8, respectively. Cocaine metabolite and ethanol were also detected.

Case 5: 63 year old male found collapsed at home having allegedly ingested 4 "Ecstasy" tablets. He was taken to hospital but later died following a cardiac arrest. 3 AM samples were analysed (between 0-2 days prior to death). An AM serum MDMA concentration of 0.44 mg/L was measured (day of death) with a corresponding PM (femoral) blood concentration of 1.14 mg/L (PM:AM ratio 2.6). Cannabinoids, cocaine metabolite and ethanol were also detected.

Overall, the PM blood concentrations were higher compared to the AM concentrations in all 5 cases (PM:AM ratio 1.1 to 2.0 in 4 cases). In one case, there also appeared to be a significant difference in the PM blood concentrations between anatomical sites (heart and femoral). Consequently, the data suggest that post-mortem blood MDMA concentrations may not accurately relate to the concentration either at the time of, or prior to death and calculations based on this assumption (e.g. dosage) should not be made.

Keywords: Ecstasy, MDMA, redistribution.

FATAL CASES DUE TO CYANIDE INTOXICATION THROUGH A LOCAL SUICIDE-FOSTERING WEBSITE

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With the rapid development of the information technology industry, new opportunities for committing suicide have appeared and become a social problem. With internet availability at most schools, companies and homes, committing suicide using the information from internet websites has been increasing. An individual opens a local suicide-fostering website that provides the information for suicide, selling poisons, recruiting members who want to commit suicide and promoting them to kill themselves together. The poisons mainly introduced on the suicide websites are cyanide salt and pesticides. Two cases related to cyanide salt were reported here. One man and two women in case 1 and three men and two women in case 2 were found dead in a motel on two separate occasions. Experimental grade potassium cyanide was also discovered in both cases. Identification and quantification of cyanide ion were performed by microdiffusion method after blood and gastric contents were collected at autopsy. Postmortem blood concentrations of cyanide ion ranged from 53.8 to 208 μg/ml in case 1 and from 5.4 to 194 μg/ml in case 2. The cyanide ion concentrations in case 2 were all over 100 μg/ml except one of the victims, where the blood concentration was 5.4 μg/ml. Based upon the history, autopsy and toxicology findings, (1) the victims were in their teens and 20s who use the internet, (2) experimental grade KCN was used in these cases while industrial grade NaCN was used in most of the previous cases, and (3) the postmortem blood concentrations in these cases were generally much higher than previous cases in Korea (0.4-74 μg/ml) and those reported in other countries (1.1-53 μg/ml). The government should strengthen education for human dignity as well as poison control in order to protect youth prone to suicide seduction.

Keywords: Cyanide, Intoxication, Internet

ARSENIC IN NAPOLEON'S HAIR: IS EXTERNAL CONTAMINATION A POSSIBLE SOURCE?

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Since the end of the 20th century, forensic toxicology experts have extensively studied the precise cause of Napoleon's death. Several toxicological studies have shown that there were significant concentrations of arsenic in Napoleon's hair, leading to the suspicion that he was poisoned with arsenic. In our work, we confirmed the presence of arsenic in the hair; however, the heterogeneity of the results between laboratories and high level of arsenic found in Napoleon's hair encouraged us to question the interpretation of these results. In a previous study by non-destructive X-ray fluorescence analysis, we could determine the presence of arsenic at high concentration in Napoleon's system. Another complicating factor was that there were no clinical symptoms of arsenic intoxication as reported by witnesses at that time. In this context, our objective was to determine if an arsenic external contamination of Napoleon Bonaparte's hair may be a plausible cause (only or associated with other factors) to explain arsenic rates of Emperor hair.

From aqueous solutions of arsenic, our protocol was to establish the quantity of arsenic capable of penetrating the hair (cut extremity protected). For this purpose, we chose varied parameters: 1) the composition of the aqueous solutions (5 different solutions), the time of contact with the hair (from 24 h to 1 month), the color of the hair (blonde, brown, and black) and the ethnic origin of the hair. Arsenic concentrations were determined on the hair, before and after four washes (five minutes successively with water, acetone, water and water sonicate), and on the liquids of washing by atomic absorption spectrometry in a pyrolytic oven with Zeemann correction (GTA 110 Zeemann, Varian®).

Major results show that: (a) Trivalent arsenic can penetrate and remain in the hair, (b) Washing the hair induces the decrease of the arsenic rate, but a significant quantity of arsenic is still firmly fixed after different treatments. (c) The time of immersion of the hair in a trivalent arsenic solution, does not change the quantity of arsenic fixed on the capillary between 24 hours and 1 month, whatever its ethnic origin. (d) The additives, likely used to prepare the disulfides bonds of the keratin for facilitating arsenic penetration, do not significantly modify the qualitative and quantitative arsenic binding, whatever the additive and the ethnic origin of the hair.

In conclusion, our study confirms that arsenic can penetrate the hair through an external source as other reports. Arsenic may remain at least partly fixed at high and toxic levels into the hair, even after full washes. Although arsenic intoxication of Napoleon cannot be excluded, our data suggests that external contamination from arsenic may provide an alternate explanation for the presence of arsenic in the hair.

Keywords: Arsenic, Napoleon, external contamination.

FATAL GHB INTOXICATION FOLLOWING RECREATIONAL USE

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Gamma-hydroxybutyrate (GHB) is encountered in biological specimens both as an endogenous neuromodulator, and as a recreational drug. Illicit GHB is currently classified as a schedule I drug in the United States due its abuse among recreational drug users. Therapeutically the drug is used for the treatment of cataplexy. Illicit doses are typically 2-4 g and the onset of action is rapid, occurring 15-30 minutes following oral ingestion. Dose dependent effects include drowsiness, euphoria, dizziness, vomiting, respiratory depression, coma and death.

GHB was isolated from biological samples using a simple liquid-liquid extraction. Trimethylsilyl derivatives were analyzed using gas chromatography mass spectrometry using positive chemical ionization (GC/MS/PCI). Deuterated internal standard and selective ion monitoring is used throughout. Samples were analyzed using an HP 5973 MSD equipped with a 30m DB-5 capillary column.

We report a GHB fatality involving a 35 year-old male who was "partying" with friends. Subjects at the party ingested unknown quantities of wine and GHB. A female companion at the party reported seeing the male alive earlier in day before she passed out. She awoke to find the individual decedent cold and stiff. Postmortem specimens were submitted to our laboratory for comprehensive toxicology testing. No alcohol or common drugs of abuse were detected in the femoral blood. A targeted analysis revealed GHB in urine, brain, vitreous fluid, femoral blood , heart blood and liver at concentrations of 1665 mg/L, 102 mg/kg, 48 mg/L, 461 mg/L, 276 mg/L and 52.2 mg/kg respectively. Concentrations of the drug in urine and vitreous fluid are extremely important in death investigations due to significant postmortem production of GHB in blood specimens. The cause of death was attributed to GHB intoxication and the manner of death was accidental.

Keywords: GHB, postmortem toxicology, fatality

INTERPRETATION OF POSTMORTEM ALCOHOL CONCENTRATIONS: A CASE STUDY

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Our laboratory performs drug and alcohol testing on approximately 2800 medical examiner cases each year. The preferred specimen for alcohol analysis was blood, and until recently, additional specimens were not routinely tested unless the submitting agency requested additional analyses. A new policy required that all positive blood alcohol measurements in postmortem cases were confirmed using an alternative specimen. Although this resulted in a 43% increase in alcohol casework, these analyses provided important interpretive information and also identified a number of unusual results that would not have been otherwise identified.

Postmortem blood and vitreous alcohol concentrations were determined in a total of 295 alcohol-positive cases. The vitreous alcohol concentration (VAC) exceeded the blood alcohol concentration (BAC) in 209 cases (71%). Blood alcohol concentrations exceeded vitreous concentrations in 81 cases (27%) and the concentrations were equivalent in 5 cases (5%). Samples that were negative in both specimens were excluded, as were cases where the confirmatory test was performed using a specimen other than vitreous fluid (e.g. urine, liver, blood from an alternative source). In casework where the VAC>BAC, linear regression analysis indicated an R^2 value of 0.958 (n=295) and a VAC approximately 18% higher than the BAC. The VAC/BAC ratio was more variable at lower BACs (<0.1 g/100mL). Although VAC/BAC ratios were more consistent at concentrations of 0.1 g/100mL and above, the overall ratio ranged from 1.01-2.20.

Of the 81 cases where the BAC>VAC, a total of 24 cases indicated no vitreous alcohol. The range of blood alcohol concentrations among these cases was widely variable (0.01 to 0.30 g/100mL). All analyses were conducted using dual column gas chromatography with flame ionization detection (GC-FID) with a reporting limit of 0.01 g/100mL for postmortem samples.

A series of case studies are used to demonstrate postmortem interpretive issues and the benefits associated with multiple specimen analysis. Cases include *in-situ* contamination of specimens due to trauma, postmortem production of ethanol, death during the absorptive phase among others. Actual case studies involving other volatile organic compounds are also discussed including isopropanol and acetone from endogenous and exogenous sources. These cases studies highlight the importance of analyzing multiple specimens in postmortem casework.

Keywords: ethanol, postmortem, blood, vitreous fluid

DETERMINATION OF FENTANYL IN POSTMORTEM BLOOD SAMPLES BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Fentanyl is a potent synthetic narcotic analgesic administrated in the form of a transdermal patch for the management of chronic pain. The authors present a liquid-liquid extraction procedure and a liquid chromatography-tandem mass spectrometry method (LC-MS-MS) for the quantitation of fentanyl in postmortem blood samples. The method was applied in a case of acute intoxication due to excessive administrated Durogesic® transdermal patch.

To a 1.0 mL aliquot of blood sample, 50 µL of internal standard solution fentanyl-d_5 (1 µg/mL) and 1 mL 1M K_2CO_3 was added. Extraction was performed with 7 mL of a mixture of n-hexane:ethylacetate (7:3, V/V). After vortex mixing and centrifugation, the upper organic layer was evaporated and reconstituted in 1.0 mL of initial mobile phase. The LC-MS-MS analysis were performed using an Alliance® 2695 system and a Quatro Micro™ mass spectrometer (Waters Milford, USA). Chromatography was achieved using a Xterra® MS C18 column 2.1 mm x 100 mm with a 3.5 µm particle size (Waters Milford, USA). A gradient elution was used with 0.15% formic acid and 0.15% formic acid in acetonitrile. The chromatographic duration was 30 minutes. The flow rate was 0.20 mL/min and the column oven temperature was set at 35°C. A 20 µL aliquot of the sample was injected. Multiple reaction monitoring (MRM) with positive ion dectection (ES+) was used for selective detection of fentanyl and fentanyl-d_5. The precursor ion and two fragment ions were selected as quantifier and qualifier, for fentanyl m/z 336.8 and fragments at m/z 187.95 and 104.4, for fentanyl-d_5 m/z 341.9 and fragments at m/z 188.0 and 104.5. Calibration was performed by addition of standard solutions to fentanyl-free blood prior to extraction. Precision, accuracy and recovery experiments were carried out. The liquid-liquid extraction procedure in combination with LC-MS-MS proved to be a good alternative compared with the more labor-intensive solid-phase extractions previously described.

The analytical procedure was applied in a forensic case: a 78-year-old woman was found death in bed, lying on het back. The external examination revealed 10 Durogesic® transdermal patches (fentanyl 100 µg/h) on the abdomen. An autopsy was carried out 2 days later. Multiple samples were taken for toxicological examination. The following fentanyl concentrations were found; 28.6 µg/L (right) and 28.2 µg/L (left) in subclavian blood, 21.3 µg/L (right) and 20.9 µg/L (left) in femoral blood, 37.6 µg/L (right) and 33.9 µg/L (left) in ventricular blood. The comprehensive systematic toxicological analysis did not reveal the presence of an other substance which contributed to the death of the woman. We concluded that death was caused by intoxication with fentanyl. The manner was presumed to be suicide.

Keywords: fentanyl, LC-MS-MS, fatality

ALFENTANIL FATAL INJECTION: A CASE REPORT

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Introduction: We report a case of fatal intoxication with alfentanil (RAPIFEN™,ALFENTA™ ampoules of 1 and 5 mg), an intravenous narcotic analgesic, chemically related to fentanyl and characterized by a very rapid onset and short duration of action. Alfentanil is used as an adjunct for surgical anaesthesia under assisted ventilation in medical units.

Case-report: A 46-year-old anaesthetist male nurse was found dead in the toilets of an hospital. A syringe was found near the body. He was suspected of narcotic theft and abuse because a lot of ampoules were missing since several months in the unit. Autopsy findings revealed injection marks on the left arm, an oedematous and inflammatory aspect of various organs and a bilateral pulmonary oedema. Biological samples (blood, urine and hair) were collected and sent to the laboratory for forensic toxicological analysis.

Methods: Screening analyses were performed by immunoassay, gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/diode array detection (LC/DAD) using standardized methods of our laboratory. GC/flame ionisation detector with headspace injection (HS) was used to determine blood alcohol concentration. Alfentanil and fentanyl were screened with GC/MS and quantification was performed with GC/MS/MS after liquid-liquid extraction with chloroform/2-propanol/heptane (33:17:50, v/v/v) and phosphate buffer (1ml, pH 9.5). Fentanyl d^5 was used as internal standard.

Results: Alfentanil was identified in blood and urine at 45.1 and 2.7 ng/ml, respectively. Blood alcohol concentration was measured at 1.32 g/l. Hair analysis revealed chronic use of alfentanil (2 pg/mg) and fentanyl (8 pg/mg). No other drugs were detected.

Adult surgical patients who received a bolus dose of 8 to 50 µg/kg alfentanil by intravenous infusion developed plasma concentrations of 200 to 1000 ng/ml. Alfentanil is capable of producing severe respiratory depression with hypotension and coma during surgical operations even with assisted ventilation and plasmatic concentrations above 100 – 200 ng/ml (1). Whereas fentanyl is reported as a commonly abused substance by healthcare professionals (2), alfentanil misuse and abuse seems exceptional (3).

Conclusion: Although the measured concentrations are therapeutic, alfentanil injection without assisted ventilation may be the cause of the death. Hair analysis demonstrated chronic abuse of alfentanil and fentanyl by the anesthetist nurse.

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Keywords: alfentanil, chronic abuse, hair, GC/MS/MS.

THE DETERMINATION OF ETHANOL ORIGIN IN FATAL AVIATION ACCIDENT VICTIMS: A MULTI-CASE STUDY

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Specimens from aviation accident victims are submitted to the FAA's Civil Aerospace Medical Institute (CAMI) for toxicological analysis. During toxicological evaluations, ethanol analysis is performed on each such case. Care must be taken when interpreting a positive ethanol result due to the potential for postmortem ethanol formation. Historically, ethanol distribution in various tissues and fluids from the same case and/or the presence of other volatile organic compounds (VOCs) at abnormal concentrations in these fluids and tissues has been employed as an indicator of postmortem microbial ethanol formation. However, these methods are not always reliable. The consumption of ethanol has been shown to alter the concentration of two major serotonin metabolites, 5-hydroxytryptophol (5-HTOL) and 5-hydroxyindole-3-acetic acid (5-HIAA). While the 5-HTOL/5-HIAA ratio is normally low, previous studies have demonstrated that the urinary 5-HTOL/5-HIAA ratio is significantly elevated following ethanol ingestion. The 5-HTOL/5-HIAA ratio is not affected by the microbial formation of ethanol, by consumption of serotonin-rich foods or by the use of SSRI's.

Recently, our laboratory has developed a single analytical approach to determine concentrations of both 5-HTOL and 5-HIAA that has provided a convenient, rapid and reliable solution to this problem. This novel methodology eliminates the need for two separate and unrelated analytical techniques, GC/MS and LC/EC, for the determination of these metabolites. The simultaneous determination of 5-HTOL and 5-HIAA in forensic urine specimens was achieved using a liquid/liquid extraction technique in conjunction with LC/MS. The ion trap MS used allowed us to perform MS/MS/MS on both 5-HTOL and 5-HIAA, and afforded limits of quantitation below 1 ng/mL for each compound. After development of this method, the previously established, antemortem, 15 pmol/nmol 5-HTOL/5-HIAA ratio cutoff was investigated and subsequently validated for use with forensic specimens.

Utilizing this newly validated method, we examined the 5-HTOL/5-HIAA ratio in urine specimens obtained from six separate fatal aviation accidents where ethanol was present, but its source was unclear. In one of these cases antemortem consumption of ethanol was indicated, even though microbial production was initially considered. After examining the 5-HTOL/5-HIAA ratio from the other five cases investigated we determined that recent ethanol consumption had not occurred. The ethanol present in these cases was due to microbial formation, and not consumption. This presentation will discuss, in depth, the difficulties in determining the source of ethanol in these six cases and the application of this novel methodology in elucidating its origin.

Keywords: Postmortem Ethanol, LC/MS, Serotonin metabolites

UNUSUAL TRAMADOL CONCENTRATIONS IN AN ACCIDENTAL DEATH INVOLVING POLY DRUG USE

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Tramadol is a synthetic opioid-receptor agonist that is used clinically as an analgesic in daily doses of 100 to 400 mg. Therapeutic concentrations in blood are reported in the range 0.1 to 0.8 mg/L. Overdoses of 500 mg and higher may cause agitation, hypertension, tachycardia and seizures whereas doses greater than 800 mg may result in coma and respiratory depression. We report a case involving unusually high postmortem tramadol (Ultram) concentrations in a 35 year-old male with a past medical history of bipolar illness, anxiety, depression and substance abuse.

Tramadol concentrations were determined using solid phase extraction (Polychrom Clin II) and gas chromatography/mass spectrometry (GC/MS) using full scan analysis with mepivicaine as the internal standard. An R^2 value in the linear range (0.2 – 4.0 mg/L) was 1.000 for tramadol. The Intraassay CV was 6.4 % using laboratory fortified independent spikes.

Initial toxicology findings of illicit drugs in femoral blood identified cocaine and benzoylecogonine at concentrations of 0.04 mg/L and 0.91 mg/L, respectively. Therapeutic prescription medications found in femoral blood included paroxetine, trazadone, tramadol and the active metabolite, O-desmethyltramadol. Quantitative analysis of tramadol revealed extraordinarily high concentrations of the parent drug. Tramadol concentrations in femoral blood, heart blood and vitreous fluid were 74 mg/L, 83 mg/L and 18 mg/L, respectively. O-desmethyltramadol concentrations in femoral blood, heart blood and vitreous fluid were 4 mg/L, 5mg/L and 1mg/L, respectively. Issues that need to be considered when interpreting toxicological findings associated with multiple drug use will be discussed.

Keywords: tramadol, overdose, postmortem

PEDIATRIC FATALITY DUE TO HYDROXYCHLOROQUINE INGESTION

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A 7 year-old African-American female was found at home unresponsive and cold approximately 4 hr after being given acetaminophen and sent to bed to rest after complaining of stomach ache and headache.

At autopsy, findings were unremarkable for clues as to cause of death. Heart blood and vitreous humor were sent to Creighton University Medical Center for forensic testing. Initially, serum and vitreous humor electrolytes, hemoglobin electrophoresis were ordered, but all results were within expected limits. The acetaminophen level was 57.6 μg/mL.

Further testing was performed using gas chromatography with FID/NPD on an alkaline extract of blood. The presence of an unfamiliar peak was noted and the Douglas County (Nebraska) Coroner was alerted to return to the home with investigators to perform a full inventory of pharmaceuticals. The inventory included numerous arthritis medications including one identified as "hydroxychlor." The parents determined with investigators present that eleven 200 mg tablets (2.2g total drug) were unaccounted for from the bottle that they thought was stored safely.

Simultaneously the laboratory was identifying the unknown GC peak as hydroxychoroquine using GC/MS with a dosage form from the hospital pharmacy as a standard.

Quantitation of the blood level of hydroxychloroquine performed at National Medical Services revealed 70 μg/mL. (NMS therapeutic range: 0.1-1.0 μg/mL) The cause of death was subsequently ruled to be acute overdose of hydroxychloroquine. The manner of death was ruled to be accidental.

According to the PDR (1) "Children are especially sensitive to the 4-aminoquinoline compounds. A number of fatalities have been reported following the accidental ingestion of chloroquine, sometimes from relatively small doses (0.75-1.0 g in one three-year-old child). Parents should be strongly warned to keep these drugs out of reach of children." Early symptoms of overdose are headache and drowsiness, and may occur within 30 minutes of ingestion. These symptoms may be followed by cardiovascular collapse, and "sudden and early respiratory and cardiac arrest". (1)

1. Physicians Desk Reference (PDR), 58th ed., (2004) p. 3031, Thomson PDR, Montvale, NJ 07645.

Keywords: Pediatric, Postmortem toxicology, Hydroxychloroquine, Fatality

LORAZEPAM AND DEATH INVESTIGATION

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The objective of this study was to evaluate the role of lorazepam (Ativan®), a benzodiazepine frequently used to manage anxiety and to sedate, in death investigations. Lorazepam has been detected with increasing frequency in death investigation cases in Washington State. A review of the literature revealed there is currently limited data on the significance of lorazepam in death investigation cases.

We reviewed death investigation cases which were positive for lorazepam at the Washington State Toxicology Laboratory between January 1998 and December 2003. The mean drug concentration found in the blood of these decedents (n=112) was 0.15 mg/L (S.D. 0.39, median of 0.03 mg/L). Concentrations ranged from <0.01 to 3.0 mg/L. There were six cases in which lorazepam was the only drug detected, one of which was ruled as a suicide by overdose, two where death was attributed to acute trauma, and three cases where the death was attributed to natural disease.

The suicide attributed to lorazepam involved a 71-year-old female, diagnosed with metastatic melanoma. Shortly before her death she discussed with her husband the possibility of 'ending her life, rather than waiting for the disease to play out.' On the day of her death the husband left the house and returned after 4 hours to find his wife in bed unresponsive. At the bedside were a partially empty bottle containing a fifth of whiskey and two empty prescription bottles of lorazepam. One of these bottles had contained 30 pills and was prescribed 7 weeks before her death. The other had contained 60 pills and was prescribed 5 ½ weeks before her death, with the instruction to take 1-2 pills at bedtime. Toxicology revealed 0.52 mg/L (peripheral blood) of lorazepam and no other drugs or alcohol. The death was certified as respiratory arrest resulting from intentional self-poisoning by lorazepam. The metastatic melanoma was listed as only a contributing factor. Unfortunately, no autopsy was performed on this subject. The coroner and doctor assigned to the case felt that the circumstances and scene indicated that the case was clearly a suicide. As a result they only obtained fluids for toxicology rather than performing a full autopsy. Therefore, the extent to which natural disease may have contributed to the death cannot be positively determined.

The trauma cases included a 59-year-old male who sustained severe injuries after falling out of bed and a 38-year-old male who died as a result of self-inflicted handgun wounds. Concentrations in these cases were 0.03 and 0.07 mg/L, respectively.

Several cases in which lorazepam ingested in combination with other drugs contributed to death will also be discussed.

Keywords: Lorazepam, postmortem toxicology, death investigation

SERTRALINE WITHDRAWAL IN A NEWBORN – COULD THIS BE A FACTOR IN THE CAUSE OF DEATH?

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A 4-day-old infant was found not breathing by her mother at about 6am. The infant was wrapped in a blanket and lying on her back on a large sectional couch near her sleeping father. The mother confirmed that the infant's face and head were several inches from her husband and were not obstructed in any way. The infant was transported to the emergency room where a heart rhythm was established and she was placed on a ventilator. The infant was later found to be brain dead and was removed from the ventilator. She was pronounced dead approximately 12 hours after the mother had first found her.

The mother received regular pre-natal care and was diagnosed with gestational diabetes. As a result she had bi-weekly testing for the last several weeks of her pregnancy. In addition she also experienced sleeplessness and had been prescribed sertraline, which she took as prescribed. The birth was induced and was an uneventful delivery resulting in a 7lb, 6oz infant. The infant was fed formula (similac) and was not breast-fed at any time. The infant was released from the hospital 2 days after she was born. Approximately 24 hours after birth the infant became irritable, screaming and crying after every feeding. The last two nights prior to her death she was awake all night in this highly irritable state.

Significant findings at the time of autopsy included epicardial hemorrhage on the anterior surface of the heart, (consistent with cardiopulmonary resuscitative efforts), and pulmonary and liver congestion.

The toxicology laboratory at MCCO received the following specimens; hospital blood (3 mL), heart blood (16 mL), cerebral spinal fluid (CSF), gastric, liver, vitreous fluid, bile and head hair. Analysis of the heart blood revealed the presence of 16 ng/mL sertraline and 113 ng/mL norsertraline, the hospital blood revealed 71 ng/mL norsertraline and analysis of the liver revealed 230 ng/g sertraline and 4000 ng/g norsertraline. Sertraline was detected in the hospital blood but the small amount of specimen received precluded its accurate measurement.

Sertraline and norsertraline quantitation was accomplished using high performance liquid chromatography (HPLC) with a C18 column and ultra violet detection with confirmation by gas chromatography/mass spectrometry (GC/MS).

A review of the literature reveals neonatal complications when selective serotonin re-uptake inhibitors (SSRI's) are used by the mother near term. Paroxetine, sertraline, fluvoxamine and fluoxetine have been associated with withdrawal symptoms in the neonate. The withdrawal symptoms are diverse but most commonly include; respiratory distress, hypoglycemia, jaundice (Costei et al. 2003) and irritability, constant crying, shivering, increased tonus, eating and sleeping difficulties and convulsions, (Nordeng et al. 2001). The symptoms usually occur after 2 days and last for an average of 10 days to one month (Salvia-Roiges et al. 2003). While the SSRI's have not been associated with teratogenic risks, it is clear that the potential for withdrawal exists – especially if the mother does not breast-feed the infant. Hendrick et al. 2003 determined cord blood concentrations of sertraline and norsertraline in 11 newborn babies whose mothers were prescribed the drug. The ranges were as follows, sertraline; less than 1 up to 14 ng/mL and norsertraline; less than 1 up to 72 ng/mL. None of these babies experienced withdrawal symptoms but it is unknown if they were breast-fed.

Based on the clinical history of the infant's feeding and sleeping difficulties, irritability and crying, and the fact that the infant was being fed formula instead of being breast fed, it is possible that the infant was undergoing withdrawal from sertraline which it was exposed to in utero. A search of the medical literature revealed no reported cases of an infant's death due to exposure to or withdrawal from sertraline but some infants exposed to SSRI's in utero required treatment for the withdrawal symptoms. The cause and manner of death was listed as undetermined. In conclusion not enough information is known to conclude that SSRI's are safe to administer to a pregnant woman, especially in the third trimester.

Key Words: Sertraline , Neonate, Withdrawal

QUETIAPINE CONCENTRATIONS IN INTENTIONAL AND ACCIDENTAL DRUG INGESTIONS

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Quetiapine (Seroquel) was introduced in 1993 as a neuroleptic agent in the treatment of psychosis. It has high affinity for serotoninergic receptor sites and less affinity for dopaminergic, adrenergic and histamine receptors. Since introduction, very few cases have been reported on the potential mortality of quetiapine overdose, alone or in combination with other drugs. We present our case series of single drug and mixed drug quetiapine overdoses from the Miami-Dade County Medical Examiner Department. The case records from the years 1998 to 2003 inclusive were searched for those cases where quetiapine was detected and quantitated in postmortem blood, gastric contents, or liver. A total of 17 cases were identified; three were excluded because no quetiapine was detected during the quantitation process, and one was excluded because a complete autopsy was not performed. Thus, 13 cases are reported in this series. The ages ranged from 28 to 55 years, with a mean age of 44 years. These cases were grouped into four categories: those where the quetiapine was the sole cause of death (Q only), those where quetiapine was related to the cause of death (QR), those where the quetiapine was unrelated to the cause of death (QUR) and those where it was unclear if quetiapine played any role in the cause of death (UNK). Two deaths were a result of solely quetiapine ingestions (Q only cases). One was a 35 year old white male with central blood, gastric and liver concentrations of 25 mg/L, 8370 mg total, and 36 mg/kg, respectively. The other was a 48 year old white female with blood, gastric and liver concentrations of 9.5 mg/L, 954 mg total, and 34 mg/kg, respectively. Two deaths in our series resulted from polydrug ingestions, where more than one agent, including quetiapine could have resulted in death (QR group). The blood quetiapine concentrations were 15.6 mg/L and 13.6 mg/L, and both had significant concentrations of at least one benzodiazepine, in addition to other agents. Four cases fell into the unknown group (UNK), where it was unclear if quetiapine contributed to the cause of death. In these cases, the mean quetiapine concentration was 1.86 mg/L, with a range of 0.34 – 2.8 mg/L. All four had other drugs detected, such as benzodiazepines, alcohol and/or cocaine. The last group, those where quetiapine clearly played no role in the cause of death, consisted of 5 cases, with a mean quetiapine concentration of 0.88 mg/L and a range of 0.27 – 1.6 mg/L. This report provides evidence that quetiapine alone can result in death , and may significantly contribute to mortality in polydrug ingestions. Also, this data may assist medical examiners and toxicologists in separating lethal from non-lethal quetiapine concentrations in a variety of circumstances.

Keywords: Quetiapine, postmortem toxicology, overdose

POSTMORTEM QUETIAPINE: THERAPEUTIC OR TOXIC CONCENTRATIONS?

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Currently, very little literature data are available on postmortem blood concentrations of quetiapine. A study was performed to establish relative guidelines for the delineation of therapeutic and potentially toxic concentrations. This study was conducted in medical examiner cases in which quetiapine was detected with routine screening methods.

Quetiapine Fumarate (SEROQUEL®) is a dibenzothiazepine derivative (2-[2-(4-dibenzo *[b,f]* [1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]-ethanol fumarate) used in the treatment of psychosis. Fourteen fatalities in which quetiapine was detected were selected for investigation.

Following liquid-liquid basic extraction, high performance liquid chromatography was used to analyze blood (peripheral and central), vitreous humor, and liver. The cases were organized into two groups: "non-drug related" fatalities and "drug related" fatalities.

A total of eight "non-drug related" fatality cases were examined. Peripheral blood concentrations ranged from: 0.26 to 0.76 mg/L (mean +/- standard deviation 0.38 +/- 0.17 mg/L); central blood concentrations: 0.23 to 0.73 mg/L (0.46 +/- 0.21 mg/L); vitreous humor concentrations: 0.08 to 0.32 mg/L (0.16 +/- 0.08 mg/L); and liver concentrations: <0.10 to 2.0 mg/kg, (0.54 +/- 0.68 mg/kg).

Six "drug related" fatalities were also examined. Peripheral blood concentration range: 3.5 to 30 mg/L (10 +/- 10 mg/L); central blood concentration range: 2.2 to 45 mg/L (16 +/- 17 mg/L); vitreous humor concentration range: 0.92 to 2.8 mg/L (1.7 +/- 0.72 mg/L); liver concentration range: 12 to 93 mg/kg, (40 +/- 29 mg/kg).

For the first time, the results of this study suggest appropriate therapeutic and toxic postmortem concentration ranges for quetiapine. Therapeutic concentrations of quetiapine are suggested to be less than 1.0 mg/L in peripheral blood and less than 5mg/kg in liver. On the other hand, concentrations indicative of toxicity are suggested at peripheral blood levels greater than 3.0 mg/L and at liver concentrations greater than 10 mg/kg.

Keywords: quetiapine, postmortem, toxicity

CAFFEINE INTOXICATION: MORE THAN JUST CREAM AND SUGAR?

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Caffeine is a mild central nervous stimulant that occurs naturally in coffee beans, cocoa beans and tea leaves. In large doses, it can be profoundly toxic, resulting in arrhythmia, tachycardia, vomiting, convulsions, coma and death. The average cup of coffee or tea in the United States is reported to contain between 40 and 100 mg caffeine. Over-the-counter supplements that are used to combat fatigue typically contain 100 mg caffeine per tablet and doses of 32-200 mg are included in a variety of prescription drug mixtures.

Caffeine was quantitatively determined in five cups of coffee purchased from local retail outlets. The total amount of caffeine per 16oz (473mL) cup ranged from 211-342 mg. Fatal caffeine overdoses in adults are relatively rare and require the ingestion of a large quantity of the drug, typically in excess of 5g. Over a period of approximately 12 months our office reported two cases of fatal caffeine intoxication. The first fatality may have occurred due to misidentification of the drug, and the second occurred after misuse of a dietary supplement.

Case #1 involved a 39 year-old female with a past history if intravenous drug use. She was found unresponsive outside a restaurant. A syringe was found near the body. Blood samples were negative for ethanol and common drugs of abuse. Comprehensive toxicology analysis using gas chromatography mass spectrometry (GC/MS) revealed a caffeine concentration of 192 mg/L in femoral blood. The cause of death was ruled as caffeine intoxication and the manner was accidental.

Case #2 involved a 29 year-old male with a history of obesity and diabetes who was admitted to the hospital with vomiting and seizures. Resuscitation efforts were unsuccessful. The family reported that he had taken a dietary supplement the day before. Toxicology results indicated a caffeine concentration of 567 mg/L in the femoral blood. The cause of death was ruled as caffeine intoxication and the manner was accidental.

Keywords: Caffeine, Intoxication, GCMS

LOW DOSE ACETAMINOPHEN DEATHS

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Acetaminophen (paracetamol) is a commonly used over the counter analgesic. It is well known that use of excessive doses can lead to the development of liver toxicity. This is mediated through the formation of the toxic metabolite N-acetyl benzoquinone imine. In adults single doses required to initiate liver necrosis is believed to start from 6 to 10 grams, which is about 12 to 20 500-mg tablets. Typically blood concentrations are well over 200 mg/L. This presentation describes acetaminophen toxicity in three women following doses within therapeutic guidelines.

Case 1. The deceased was a 28 year old female of age with a history of an eating disorder, depression and alcoholism. While in hospital for an unrelated condition she became drowsy, weak, ataxic, and had slurred speech. She died some hours later. She had received about ten tablets over the previous 3-4 days. Her maximum acetaminophen concentration was 38 mg/L.

Case 2. The deceased was a 21-year-old healthy woman. She developed a laceration after falling on glass. Three days later she presented to hospital with severe headache associated with vomiting and mild photophobia. A few days later she was weak, was vomiting and had a high heart rate. She was diagnosed with hepatic encephalopathy and her acetaminophen concentration was 46 mg/L. She had taken about 10 acetaminophen tablets over a few days. Other causes of liver necrosis were excluded by exhaustive toxicology, serology and virology testing.

Case 3. This 45-year old woman had undergone surgery for an obstructed bowel. A few days later she was confused and agitated. This worsened until her death on day six. The day previously she had been diagnosed with liver failure. Her maximum acetaminophen concentration was 46 mg/L. She had been given one gram acetaminophen two to three times daily for several days prior to her death.

All cases showed typical liver necrosis at post-mortem and had clinical changes consistent with acetaminophen toxicity. None of the cases had circumstances suggesting recent abuse of acetaminophen. The low acetaminophen concentration and absence of other causes of liver necrosis suggests that some persons may be sensitive to this drug, perhaps because of low glutathione concentrations due to diet or genetic factors and/or were genetically predisposed to the development of the toxic metabolite. A full account of the toxicology and post-mortem findings will be presented.

Keywords: acetaminophen, low-dose, toxicity, post-mortem

INTERPRETING ANTIHISTAMINE LEVELS IN POST-MORTEM BLOOD; ESTABLISHING INCIDENTAL AND CONTRIBUTORY RANGES FOR EVALUATION PURPOSES

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Over the last ten years, the Food and Drug Administration has approved numerous prescription antihistamines safe for over-the-counter (OTC) distribution. Antihistamines are a broad class of drugs, frequently taken to offer symptomatic relief from colds, sinus congestion, allergies, and are also a class of drugs frequently abused.

In forensic toxicology, post-mortem (PM) redistribution of drugs complicates the interpretation of results. Many of the reference materials available are for clinical interpretation, and extrapolating PM levels for comparison to clinical values is not ideal. The substantial volume of casework at the Los Angeles County Department of Coroner (LACDOC) Laboratory provided us the ability to establish applicable PM blood level ranges for interpretation of the first generation antihistamines by a query of the LACDOC toxicology database over a three-year period. First generation antihistamines are easily detected using a basic liquid-liquid extraction with an acidic back extraction. Diphenhydramine, chlorpheniramine, doxylamine, promethazine, and hydroxyzine are commonly detected at the LACDOC by GC/NPD.

A brief summary of the query results and interpretation are as follows:

	Post Mortem Central Blood Levels, mg/L				
Categories	Diphenhydramine (471 cases)	Doxylamine (87 cases)	Promethazine (136 cases)	Hydroxyzine (44 cases)	Chlorpheniramine (126 cases)
Published Clinical Therapeutic Drug Levels	~ 0.11	~ 0.14	~ 0.10	~ 0.08	~ 0.02
Determined "Incidental" Drug Levels	0.01 – 0.70 81% (383)	0.01 – 0.70 84% (73)	0.01 – 0.30 74% (101)	0.01 – 0.50 73% (32)	0.01 – 0.30 96% (121)
Determined "contributory" to the cause of death	> 0.70 17% (80)	> 0.70 15% (1)	0.31 – 0.0.90 16% (21) and > 0.90 10% (14)	0.51 – 1.1 18% (8) and > 1.1 9% (4)	> 0.30 4% (5)
Determined as the "sole cause of death"	> 19 2% (8)	> 132 1% (1)	--	--	--

The query results were categorized as follows:

- Incidental - Levels that represent over 70% of the quantitated cases for a particular drug, and correspond to therapeutic dosing
- Contributory - Levels that were greater than therapeutic and could not be ruled out as a contributing cause of death
- Sole Cause of Death – Due to the drug alone, not influenced by any other drug

This study determined PM therapeutic central blood levels for five, first-generation antihistamines and central blood values that indicated the drug was a contributing factor in the cause of death. The Los Angeles County Coroner Laboratory continues to evaluate the cases where levels are greater than the "contributing" category and may have been the sole cause of death, if other weren't drugs present.

Keywords: Antihistamines, Interpretation, Post-mortem blood levels

PHARMACOGENOMICS AS MOLECULAR AUTOPSY: GENOTYPING *P450 2D6, 2C9* AND *2C19* USING PYROSEQUENCING™ FOR METHADONE CASES

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Pharmacogenomics, the study of the impact of heritable traits on pharmacology and toxicology, has the potential to explain the relationship between drug administration, drug metabolism and adverse drug reactions. Genetically, single nucleotide polymorphisms (SNP's) are single base-pair changes in the DNA sequence of a gene. Base deletions or substitutions may result in a change in the amino acid sequence of the polypeptide. These heritable mutations have the ability to change the structure and specificity of the enzyme to the extent that it may not metabolize the drug in question. Depending on the variant alleles present, an individuals' metabolic rate may be phenotypically described as poor (PM), intermediate (IM), extensive (EM, normal), or ultra-extensive (rapid). PM's have a high risk of adverse effects which can potentially be fatal, while IM's have a metabolic rate between PM's and EM's.

In Forensic Pathology/Toxicology, molecular techniques can determine the genotype of a decedent and may aide in the determination of the cause and manner of drug related deaths. Methadone, for example, is metabolized in the liver by cytochrome *P450 (CYP) 1A2, 3A4, 2D6* and, to a certain extent, *2C9* and *2C19* enzymes. All of the genes encoding these enzymes are polymorphic. In this study we establish the technical feasibility of genotyping for known mutations in *CYP2D6* (**3*, **4*, **5*, **6*, **7* and **8*) as well as *CYP2C9* (**2*, **3*) and *CYP2C19* (**2*, **3*, **4*) using Pyrosequencing™.

Twelve frozen, archived, blood samples from the Milwaukee County Medical Examiners Office (MCMEO), were selected as part of a multi-center retrospective analysis of cases from June 2002 to December 2002, which was approved by Medical College of Wisconsin IRB. These cases listed methadone as a contributing factor in the cause of death. After DNA extraction, PCR was performed and amplified product was then interrogated for the presence of specific SNP's using a Pyrosequencing™ PSQ96MA. Briefly, a sequencing primer is hybridized to a single stranded PCR product, and incubated with enzymes, DNA polymerase, ATP sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate (APS) and luciferin. A deoxynucleotide triphosphate (dNTP) is added to the reaction. If incorporated, a release of pyrophosphate occurs that is equimolar to the number of incorporated nucleotides. ATP sulfurylase quantitatively converts PPi to ATP in the presence of adenosine 5' phosphosulfate. This ATP converts luciferin to oxyluciferin and generates visible light detectable by a CCD camera and evident by a peak in the Pyrogram™. Each light signal is proportional to the number of nucleotides incorporated. Apyrase degrades the unincorporated dNTP's and excess ATP prior to the addition of another dNTP. Software correlates each Pyrogram™ to a reference sequence and genotype determinations are made. This platform has been previously validated for clinical samples, with results showing 100% concordance with other platforms including conventional PCR with RFLP analysis, direct sequencing using an ABI 3100, and rapid-cycle PCR with fluorescent melting curve analysis using a Roche LightCycler.

Genotyping results for *CYP2D6* indicated 8 cases (66.6%) of EM's, 3 cases (25%) of IM's and 1 case (8.3%) of a PM. For *CYP2C19* SNP's, 8 samples (66.7%) were found to be EM's and 4 samples (33.3%) were found to be IM's. No mutations (0%) were detected for *CYP2C9* in any sample. These percentages are comparable to the expected prevalence ranges found in the general population. In this preliminary study, we demonstrate the feasibility of genotyping forensic samples for multiple SNP's associated with methadone metabolism. These protocols will be used in a large (n > 2000) multi-centers methadone study.

Keywords: Pharmacogenomics, Methadone, Pyrosequencing

A MULTI-CENTER STUDY OF PHARMACOGENOMICS AS AN ADJUNCT OF MOLECULAR AUTOPSY FOR METHADONE DEATH CERTIFICATION – PRELIMINARY FINDINGS OF DATA ACQUISITION AND MULTIPLEX GENOTYPING *CYP 450 2D6, 2C9* AND *2C*19 BY PYROSEQUENCING™

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Methadone is used in the treatment of heroin addiction and as an analgesic for pain management. Recently, there has been an increased incidence of methadone intoxication due to abuse and diversion. One of the contributing factors to drug therapy and toxicity is genetic variation. With the Human Genome Project near completion, genomic medicine based on genetic profiles has been proposed for personalized medicine – for drug treatment and other therapeutic approaches. As an extension of genomics medicine in forensic science, a previous study examined the potential contribution of genetic variations in methadone death (J For Sci. 2003;48:1406-15). The allelic frequency of wild-type, defined for that study as non- *CYP 2D6 *3, *4* and **5*, was 71.4%, lower than that of living controls 82.7%. Due to the limited number of cases (n =21), the study was not adequately powered with sufficient cases to reach statistical significance. Studies for oxycodone and antidepressants also yielded a similar lower prevalence. Further, there is increasing understanding of methadone metabolism, readily characterized as multi-pathways with multi-enzymes (CYP 3A4, 2C9, 2C19, and 2D6). Some of the genes encoding these enzymes are polymorphic. Hence, a multi-center study was designed to enroll an adequate number of methadone cases to evaluate the potential contribution of genetic variation to methadone/related deaths – Pharmacogenomics as an aspect of Molecular Autopsy. The study was initiated with several planning sessions to consider all the key co-variables and risk factors. In the fall of 2003, the following medical examiner/coroner offices and personnel formed the Forensic Pathology/Toxicology Pharmacogenomics Methadone Study Group (FPTPMSG): Milwaukee - Wong, Jentzen, Gock, Jannetto, Schur, Sahin, Frolov, Rogalska, Cleveland/Cuyahoga – Jenkins, Balraj, Detroit/Wayne – Hepler, Isenschmid, Schmidt, Miami/Dade – Hearn, Uhlin-Hansen, New Hampshire - Wagner, Andrew, New Mexico – Kerrigan, North Carolina - Winecker, Ropero-Miller, San Diego - McIntyre, San Francisco – Karch, Lemos, British Columbia/Canada– Langman, Washington, DC – Couper, and Washington – Logan, Gordon. In addition, drug-drug interaction interpretations will be performed by Moody (Univ. Utah) and secondary case reviews by Jortani (Univ. Louisville). The study period is 2002 and 2003, with some offices providing samples before and after for trending studies. The Medical College of Wisconsin (MCW) approved an " Umbrella IRB", with subsequent endorsement by the participating offices. Inclusion criteria are: cases certified with methadone toxicity, methadone related and methadone present. For the latter cases, each office would seek informed consent by decedent's family. Whole blood samples were mailed to the MCW Pharmacogenomics Lab for multiplex genotyping for *CYP 2D6 *3, *4, *5, *6, *7, *8., 2C9 *2* and **3.*, and *2C19*2,*3* and **4* by Pyrosequencing™ (Clin Chem. 2003;49:A12). By using a combination of Microsoft ACCESS and Excel database programs, case history and toxicology results were entered, followed by statistical analysis of transformed Excel data. At this preliminary stage, the study demonstrated the feasibility of coordinated planning and initial data entry and transfer to the MCW via Internet by 5 centers. The projected total caseload for FPTPMSG is more than 1900. For a limited number of samples (n=22), the following variants were identified in 14 samples: 1 homozygous for *CYP 2D6*4*, and the number for heterozygous variants – 2 for *2D6*4* and *2C19*2.*, 1 for *2D6*3/*4.*, 2 for *2C9*2*, 1 for *2C9*3*, 4 for *2C19*2*, 1for *2D6*3*, and 2 for *2D6*5*. These preliminary results indicate that the multi-center study is proceeding on schedule, albeit with the expected degree of logistic and technical difficulty.

Key words: methadone, pharmacogenomics, death

2,4-DINITROPHENOL (DNP): AN UNUSUAL FATALITY

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The decedent was a single 28-year-old male college student who resided with a roommate. His roommate found the decedent, with a fever, in a tub of ice. The roommate transported him to a local fire department, from where he was then transported to a local hospital. The decedent was conscious upon admission, but was soon pronounced dead despite resuscitative efforts. The decedent's roommate informed hospital staff that the decedent had been taking bodybuilding supplements. Bottles of medications and other materials found at the scene were brought to the Medical Examiner's Office. These included steroids, ephedrine, thyroid hormones, a yellow powder and a syringe.

Autopsy showed a young muscular jaundiced Caucasian male with left ventricular hypertrophy of 1.6 cm, multiorgan congestion, and severe pulmonary edema. The liver was of a soft mottled purple and brown appearance. There was 150 mL of blood in the stomach, signs of hemorrhagic gastritis, and evidence of CPR (fractured sternum and anterior rib fractures). Autopsy also showed severe cerebral edema with uncal herniation and other evidence of medical therapy. Microscopically, the liver showed severe sinusoidal congestion and subtle centrilobular hepatocyte necrosis. No other significant macroscopic or microscopic findings were noted.

Routine toxicological screens (alcohols, drugs of abuse, basic drugs, acidic drugs) were conducted on the antemortem specimens obtained from the admission. The only therapeutic drugs detected were diphenhydramine (0.13 mg/L) and lidocaine (<0.05 mg/L). Specific analyses for steroids (postmortem urine) and ephedrine (antemortem blood) were negative. Analysis of the yellow powder by gas chromatography/mass spectrometry identified the powder as 2,4-dinitrophenol (DNP). Subsequent analysis of the postmortem urine (Toxi-Lab, confirmed by gas chromatography/mass spectrometry), then detected the presence of DNP. The acid drug screen (HPLC) also detected an unknown compound in the antemortem blood that was later identified as DNP. DNP was then quantitated by HPLC in the antemortem blood and in the following postmortem specimens: blood (peripheral), serum (peripheral), liver, urine, bile and gastric contents.

DNP is primarily used commercially in the production of dyes, wood preservatives, photographic developers, explosives and pesticides. In the 1930's DNP was used extensively as a diet pill. DNP effectively increases the basal metabolic rate by inhibiting the synthesis of ATP in the mitochondria. This action also results in increased sweating, breathing rate, heart rate, weight loss and a marked increase in body temperature. In 1938 the U.S. Food and Drug Administration banned the use of DNP due to its harmful effects, which included cataracts, liver damage, and in some cases death.
This is the first report of a fatality associated solely with DNP that describes the detection of the compound in antemortem blood and postmortem tissue concentration distribution.

Keywords: 2,4-Dinitrophenol, postmortem, fatality

GHB CONCENTRATIONS IN A VARIETY OF ALCOHOLIC AND NONALCOHOLIC BEVERAGES AND LIQUID FOOD PRODUCTS

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Casework indicated the presence of small amounts of GHB in samples of Cabernet Sauvignon and Steel Reserve beer, which according to previous reports could be expected, at least in wine. Due to these results a variety of samples of different alcoholic, nonalcoholic beverages, and fermented liquid food products were analyzed for the presence of GHB following the method previously described by Couper and Logan* using smaller volumes of sample (100-200 mcL). The limit of detection was 0.25 mg/L. A total of 108 samples were tested in duplicate.

A number of the samples analyzed were positive for GHB although at very low concentrations. In general, the concentration of GHB was higher in red wines (2.00-23.00mg/L; n=11) than in white wines (0.65-9.53 mg/L; n=13). Of all the different kinds of vinegar tested (n=5) only white vinegar was negative for GHB while rice wine, red wine, balsamic and apple cider vinegar were positive (0.83-11.25 mg/L). The GHB concentration in beer (<0.25-2.10 mg/L; n=29) was lower than it was in wine. For the different kinds and brands of beer tested, there appears to be a correlation between the GHB concentration and the ethanol content rather than with the type of beer analyzed. Although the sample size was small (n=3), the home brewed beers which typically have a higher alcoholic content also had the higher GHB concentration (1.71-2.10 mg/L). The GHB concentration was either not detected or < 0.50 mg/L in the distilled alcohol samples tested (n=23). The GHB concentration in the liqueurs tested varied from not detected to 4.20 mg/L (n=11). A variety of other non distilled alcoholic drinks, such as sherry, vermouth, and champagne, showed intermediate concentrations of GHB (1.88-6.68 mg/L; n=5). Neither of the two malt beverages contained GHB above our cutoff. GHB was detected in grape juice (n=2) but not in apple juice. GHB was in noni juice (Morinda citrifolia, (0.73 mg/L) , but not in the tea from the noni leaves. No GHB was detected in the teriyaki sauce tested (n=1). The three different kinds of soy sauce tested were positive for GHB (2.79-18.10 mg/L).

We conclude that although some of these products contain GHB, a minimum of 210 L of these liquids would need to be consumed in order to ingest a 5 g dose of GHB.

Couper and Logan*(2000) JAnalTox 24: 1-7

Key Words: GHB, alcoholic beverages, food products.

THE USE OF CLUSTER ANALYSIS TO ESTABLISH A REFERENCE RANGE FOR BETA-HYDROXYBUTYRATE CONCENTRATIONS IN POSTMORTEM BLOOD AND ITS APPLICATION TO THE INVESTIGATION OF SUDDEN UNEXPECTED DEATH IN PROBLEM DRINKERS

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Alcoholic ketoacidosis is a relatively common cause of death in problem drinkers. (1) It should be considered in every case of sudden unexpected death in a problem drinker, particularly if hepatic steatosis (a fatty liver) is present and "routine" toxicology screening is unrevealing. An important clue may be a trace of acetone apparent on the ethanol assay chromatogram in association with a low or zero alcohol concentration. If the toxicologist or pathologist have any degree of suspicion that the death might be associated with alcoholic ketoacidosis, then beta-hydroxybutyrate should be measured in post mortem blood.

Whilst the diagnosis of alcoholic ketoacidosis is straightforward when the beta-hydroxybutyrate concentration is grossly elevated in post mortem blood, the interpretation of the borderline result may be difficult. In life, clinical laboratories typically quote the upper limit of normal for the fasting plasma beta-hydroxybutyrate concentration as from 300 to 600 micromoles per litre. Unfortunately, whilst in principle it should be a simple matter to establish a reference range for the beta-hydroxybutyrate concentration in post-mortem blood, in practice, for those working in England and Wales, this is fraught with difficulty. Most post-mortem examinations are non-consensual, carried out under the direction of the coroner. In the analysis of samples collected during such post-mortems, it is only permitted to carry out analyses directed at establishing who the deceased was and where and when he came to his death without the specific permission of the coroner and the relatives of the deceased. Whilst a "black letter" interpretation of the present law is arguably more liberal, those who have retained specimens obtained at a coroner's post-mortem for research or teaching have been subject to considerable public criticism in the UK. (2). The practicalities of obtaining permission from the relatives of the deceased who is about to be subject to a coronial post mortem examination are daunting. If legislation currently before the UK Parliament becomes law in its present form, then the researcher who analyses a post mortem blood sample without permission will be liable to prosecution with a possible penalty of up to 3 years in prison on conviction. (3)

Consequently it is necessary to make the best possible use of the data obtained when investigating any particular case to inform the interpretation of future cases. One approach that we have found useful is to use cluster analysis of all of the data we have obtained in the investigation of possible cases of alcoholic ketosis to delineate normal, equivocal and elevated beta-hydroxybutyrate concentrations in post-mortem blood. We further believe that the technique may be of more general application in the analysis of post-mortem toxicology data. To illustrate the technique we present the analysis of our data in relation to the investigation of alcoholic ketoacidosis and, more briefly, possible overdoses of diphenhydramine and tramadol.

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Keywords: alcoholic ketoacidosis, beta-hydroxybutyrate, cluster analysis

POSTMORTEM BLOOD ALCOHOL RELIABILITY: FEMORAL SAMPLING?

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The analysis of blood for alcohol is one of the more fundamental analysis undertaken by a forensic toxicology laboratory. The sources of error in the laboratory are well documented and international standards of laboratory practice (AS/ISO 17025) have been developed to safeguard the analytical processes, for any analysis. Discrepancies in the alcohol levels in post mortem specimens from a recent vehicle accident death resulted in a detailed review of all aspects of the post mortem process. In this case two femoral blood samples gave alcohol levels of 0.310g/100mL but a vitreous humor sample had no detectable alcohol.

A number of papers have been published on the importance of the source of blood samples used in quantitative alcohol and drug analysis. Errors due to contamination, dilution and "post mortem redistribution" are cited. The conclusion is that peripheral samples should be used and best source is the femoral vessels. The recommended procedure for post mortem femoral blood sampling is reviewed. The sampling point is normally within the pelvic girdle and it is clear that poor adherence to correct procedures can result in adventitious contamination or a non-representative blood sample. Traumatic deaths present the greatest risk of contamination. The Laboratory receives approximately 3000 toxicology cases per year and a review of 2 years showed the median volume of preserved blood submitted from coronial cases was 20mL.

Mathematical modelling demonstrated that 200µL (4 drops) of an alcoholic spirit in the pelvic cavity could result in blood alcohol levels of 0.300g/100mL, if allowed to contaminate a 20mL blood sample. Several tables are presented of various collected blood volumes and potential contamination factors.
The reliability and predictability of comparing blood and vitreous humor alcohol levels was also tested. A set of 33 blood alcohol levels greater than 0.200g/100mL were compared to their respective vitreous humor alcohol levels. The regression equation was $y=1.2734x-0.0451$, the R^2 was 0.9488. This result was similar to other reports and demonstrated the value of this comparison as well as the improbable results for the reviewed case.

The review did not categorically identify the source of error in this case but the work has highlighted the ease of introducing significant errors in blood alcohol analysis. It has also supported the strong correlation between blood alcohol and vitreous humor. This observation makes this specimen particularly useful in contentious toxicology cases and should be taken in matters involving severe trauma. The growing convention in forensic toxicology is to use femoral blood samples for quantitative analysis, is endorsed but correct sampling procedures must be followed by pathologists.

Keywords: Blood alcohol, error, femoral, vitreous humor.