ABSTRACTS

1

Concentrations of Morphine and its Glucuronides Among Fatally Poisoned Heroin Addicts and Patients During Oral Morphine Therapy

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Morphine (MO), morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) were determined in blood, CSF and vitreous humor of dead heroin addicts (12 cases) and in blood samples taken from four patients undergoing oral morphine therapy. The analytes were determined by means of HPLC with coulometric detection, tuned for particular analytes, after solid phase extraction. The recovery was 80% for MO and 60% for M6G and M3G.

The following molar ratios in blood were found:

Drug	M6G/MO	M3G/MO	M3G/M6G
Heroin, I.V.	1.5 ± 1.3 0.1 - 2.8	2.7 ± 3.3 0.1 - 11.0	2.5 ± 2.0 0.2 - 6.2
Morphine, P.O.	0.3 - 1.2	2.9 - 11.2	7.0 - 33.5

The concentrations and the ratios of M3G/MO, M6G/MO and M3G/M6G were compared with the available data observed among patients after chronic and acute morphine application by different routes.

The molar ratio of M6G/MO appeared to be higher in heroin fatalities than observed during MO therapy. In one case (suicidal I.V. infusion of heroin), very low levels of glucuronides were found.

Key Words: Morphine-3-Glucuronide, Morphine-6-Glucuronide, Heroin Addicts.

2

Toxicological Findings in Two Children, One with Multiple Hospital Admissions: "Münchhausen's Syndrome by Proxy"?

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A fifteen month old white female underwent several admissions and E.R. visits to a hospital, with a variety of toxicological findings upon the final admission which resulted in death with a clinical diagnosis of atypical seizure disorder. Autopsy findings, including neuropathology, failed to demonstrate any overt disease processes. Serum obtained 36 hours following the last admission and prior to death, demonstrated the presence of acetaminophen, (6.6 µg/ml), whereas a urine specimen obtained at approximately the same time contained acetaminophen, caffeine, phenobarbital and thiopental. The latter drug had been used for intubation and the phenobarbital was a metabolite of mephobarbital previously prescribed for seizures. Approximately 3 months later, an 8 month-old sibling presented at the E.R. with the same clinical history as the deceased child. A stool specimen was submitted from this infant 2 days after admission for toxicological analysis. The specimen contained numerous tiny seeds which when submitted to GC/MS analysis were found to contain lorazepam and temazepam. The potential role of these benzodiazepines from a natural product will be discussed along with circumstances regarding the parent's role in the children's clinical symptoms.

Key Words: Münchhausen's Syndrome by Proxy, Multiple Hospital Admissions.

3

Deaths Associated with Benzodiazepines

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Benzodiazepines are among the most commonly detected group of drugs in decedents reported to the coroner. Benzodiazepines are rarely associated directly with drug-related deaths, in most cases the presence of other more toxic drugs are involved in the death, with benzodiazepines usually contributing a minor role in eliciting toxicity.

I describe 20 deaths over a 5-year period in which both the pathologist and coroner have concluded that benzodiazepines contributed directly to the death. A full postmortem examination was conducted on all cases with the exception of one case which was an inspection and report due to an objection to autopsy on religious grounds. All cases involved a full toxicological work-up of submitted specimens. Benzodiazepines were quantified by gradient HPLC (McIntyre IM et al, J Anal Toxicol, 1993; 17: 202-7).

Benzodiazepines represented in these deaths were: temazepam (n=6), flunitrazepam (n=8), nitrazepam (n=7), oxazepam (n=6), diazepam (n=4), flurazepam (n=1), bromazepam (n=1) and clobazam (n=1). In most cases (n=13) more than one benzodiazepine was detected. No other drugs were detected which would have contributed significantly to the deaths, including alcohol, which was not detected in any of the cases. Suicides represented 14 of the cases, in the other cases it was not clear from the circumstances whether death occurred from suicide or just misuse or abuse of benzodiazepines. The age range of the deaths were 22 to 85 years, mean 56 years and median 62 years. Nine cases were females.

These deaths indicate that the misuse of benzodiazepines can cause death and that no one benzodiazepine is necessarily spared from this potential. Consequently, a thorough postmortem examination for benzodiazepines would therefore be deemed necessary in all cases to exclude the presence of these drugs.

Key Words: Pharmacology, Benzodiazepines, Deaths, Suicide.

4

Quality Control in Forensic Toxicology Using Postmortem Blood and Urine Samples: Play the Real Game!

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An original concept for quality control in forensic toxicology was developed by the Medico-Legal Institute in Strasbourg, France. Blood and urine were sampled during the autopsy of a 25-year-old drug addict, who died under circumstances suggesting a drug overdose. After homogenization, aliquots of blood (ca. 12 mL) and urine (ca. 7 mL) were mailed to 9 French forensic toxicology laboratories who were empowered to perform toxicological appraisements for Courts of Justice. The participants had to assay the samples and answer the following questions:

- What compound(s) did you identify? (qualitative trial)
- At what concentration(s)? (quantitative trial)
- What are your conclusions about the cause of death? (interpretation trial)

The following compounds were identified: opiates (9 labs/9), cannabinoids (9/9), benzodiazepines (8/9), trimeprazine (7/9), cyamemazine (6/9), mianserine (4/9); no presumed false positive was reported, except for cyanide (1 lab). Large interlaboratory variations in the quantitative results were observed, especially with the phenothiazines (cyamemazine: 5.2 to 150.0 ng/mL blood). Morphine levels in blood were in the range 295 to 960 ng/mL. Only 6 labs could identify (and 2 quantify) 6-MAM in urine, allowing the cause of death to be ruled a heroin overdose associated with the ingestion of several pharmaceutical preparations.

The advantages (general-unknown screening in "difficult" biological matrices) and limitations (absence of a reference laboratory) of such a new design of quality control under "real" forensic conditions are compared to classical systems.

Key Words: Quality Control, Forensic Toxicology, Post-mortem Samples.

5

Method Specific Quality Assurance Concept for Drugs-of-Abuse Testing in Urine: Preliminary Results of a Second Collaborative Study of 18 Participating Laboratories from EU-Member States and the United States

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A screening method for the detection of drugs of abuse in urine was modified for quality assurance (QA) purposes in the authors' laboratories. After a first evaluation (1) and a first collaborative study (2), the project has now been extended to other laboratories from EU-Member States and one HHS accredited laboratory from the US. The results of this project with 2 samples, A/B, were as follows:

Drug	Spiked (ng/mL)	Mean Found (ng/mL)	CV %
Amphetamine	0/760	n.d./813	- /35.6
Methamphetamine	0/1360	n.d./1107	- /44.8
Phencyclidine	0/34	n.d./32	- /23.6
Benzoylecgonine	110/180	109/160	13.2/11.1
Morphine	525/225	451/168	35.4/37.4
Codeine	280/170	260/162	22.0/14.0
THC-COOH	90/42	81/33	32.0/21.0

1. M. R. Moeller, M. Hartung and R. Wennig, A New Concept of Quality Control in Drugs-of-Abuse Testing, AAFS Meeting, New Orleans, LA 1992.

2. R. Wennig, M. R. Moeller, M. Hartung and M. Flies, Collaborative Study Results of 9 Participating Laboratories from EC-Member States to Evaluate a Method Specific Quality Assurance Concept for Drugs-of-Abuse Testing in Urine by Solid Phase Extraction and GC-MS Quantification, in Forensic Toxicology, TIAFT, Leipzig 1993: "Contributions to Forensic Toxicology" R. K. Mueller, Ed., Molina Press, Leipzig, 1994.

Key Words: Quality Assurance, Drugs-of-Abuse Testing, Collaborative Study.

6

Blind Performance Testing for Drugs of Abuse - An Overview of Ten Years Experience

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For the past 10 years, nearly 100,000 specially prepared performance test (PT) samples have been submitted blind to 25 drug testing laboratories in the U.S. Frozen samples were sent over time to over 150 collection sites for eventual submission to the laboratories to ensure they would appear as routine specimens. The PT samples were prepared in large lots to ensure that each laboratory received identical samples over time. This permitted inter- and intra-laboratory comparisons over several months. From 1984 through 1988, performance across 10 laboratories covered during this period ranged from 74% – 100% ((correct results/number of samples tested) X 100). With the implementation of laboratory certification under the HHS Mandatory Guidelines in 1988, performance has gradually improved, with the lowest scores averaging 88%. Errors were primarily false negatives, with very few false positives after 1988. The records for blind PT testing are reviewed periodically by unannounced inspections of the laboratories. This assures that false negatives are due to appropriate causes, e.g., a missed cutoff, rather than other more serious technical problems or administrative errors.

In 1993, three laboratories in Canada and three in Great Britain and Europe were added to the blind PT program. Because Canada has adopted accreditation standards similar to the U.S. Guidelines, performance has been in the 98% – 100% range. Similar results have been obtained for the carefully selected European laboratories, although an unexplained false positive result for pholcodine was reported. These laboratories are inspected annually using modified versions of the HHS Guidelines laboratory inspection criteria. The use of blind PT samples to monitor laboratory reliability has proven to be a highly effective tool.

Key Words: Performance Testing, Drugs of Abuse.

7

Telematic Proficiency Testing Program on Psychoactive Substances

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Quality Control has long been recognized by manufacturing, cultural, scientific and health industries to be essential. Toxicology laboratories can achieve maximum reliability both by promoting internal and inter-laboratory quality control checks suitable for the techniques used and by adhering closely to the quality control guidelines set by the most highly qualified international agencies and societies. Current legislation in Italy still lacks compulsory implementation of Proficiency Testing Programs in the sector of drugs of abuse. The only initiative taken on a national level in the last decade is the "Drugs of Abuse" Quality Control program, co-ordinated by the University of Padova. This initiative, set up to promote a scientific and cultural service, continued until 1987 and, in the form of annual trials, until 1992.

Supported by experience in this field, the Centre of Behavioural and Forensic Toxicology (CBFT) of the University of Padova has promoted a new long-term Proficiency Testing Program for psychoactive substances, based on an innovative computer-linked telematic network of laboratories. The Program, assuring participants' anonymity by a coding system, offers laboratories three options: two for qualitative results and one for quantitative results. Batches of urine samples, validated by "Reference Laboratories", are sent every 3 months by the most prompt delivery method.

The 190 currently participating laboratories receive, in real time, reports containing: 1) qualitative-quantitative composition of the last batch of samples; 2) correct results, false positives, false negatives, "major" and "medium" errors, percentages of error and percentages of weighted error, produced on the last batch of samples; 3) error frequency in analysing each class of substances in the last batch of samples; 4) degree of efficiency on the last batch of samples and on batches during the current year; 5) average degree of efficiency of all participating laboratories on their last batch of samples; 6) time trend of the efficacy index of the Program, calculated according to the degree of difficulty encountered and the index of overall error by all laboratories on each batch of samples; 7) frequency of use of each Isolation/Pretreatment Procedure and Analytical Technique by all laboratories on the last batch of samples; and 8) frequency of accurate results obtained with each Analytical Technique by all laboratories on the last batch of samples.

In order to improve knowledge and specialized background, the Program supplies scientific information (updated documentation on analytical methods and forensic topics, annual workshop and reports) and technical services, including analysis and certification of specimens presenting exceptional analytical difficulties in the search for psychoactive substances. The lowest error rates were encountered in the analyses of opiates (2.0%) and methadone (1.2%), the highest in the analyses of benzodiazepines (5.3%) and barbiturates (11.5%). The most commonly used analytical screening techniques were EMIT and ADx, whereas the most employed coupling of techniques was EMIT + GC/MS followed by ADx + REMEDi. The highest percentage of correct results using a combination of techniques was obtained by the EMIT-GC/MS combination followed by the ADx-REMEDi combination. The average degree of efficiency obtained by the participating laboratories was 96.45%. Thanks to the telematic network, data were made readily available also to all the participating laboratories in real time. Developments in methodology and results since 1980, presented here, confirm that Proficiency Testing Programs with a strongly educational orientation should be set up on national and international scales.

Key Words: Psychoactive Substances, Proficiency Testing, Telematic Network.

8

Strategies for Surveys on Drugs of Abuse Testing Reliability: The European Approach

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Several strategies are possible to obtain information on methodological aspects, quality of results and their interpretation in urine drug testing. The protocol was used in a two-step survey of more than 200 laboratories from the twelve Member States of the European Union. Main objectives were to gain information on the present procedures and to monitor the effect of some improvement measures.

a) First step (developed in 1993).

Samples of sterile spiked urine containing several drugs/metabolites (contents verified by reference laboratories) including amphetamines, opiates (including methadone), cocaine and cannabinoids were distributed to laboratories. Results were collected by means of a comprehensive data collection form. In addition to representative data on detection, confirmation and quantification of the drugs, the structure of the form afforded interesting knowledge about use or misuse of analytical cutoffs, and the toxicological criteria used to determine final results and many additional analytical aspects.

b) Second step (developed in 1994).

In order to optimize laboratory performance, the following materials were distributed: lyophilized reference urines, drug standard solutions and deuterated drug standards. Capabilities of laboratories will be monitored after the analysis of spiked urines (similar concentrations to the first step) and also real clinical urines.

The described strategy appears highly valuable as a comprehensive approach to assess multi-laboratory drug testing capabilities.

Key Words: Drug Testing Surveys, Reference Materials, European Approach.

9

Codeine Concentrations in Plasma Versus Aged Whole Blood

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Codeine is frequently prescribed as an analgesic and antitussive and is commonly found in post-mortem analysis. We analyzed plasma and aged whole blood samples for codeine and metabolites and compared the results to determine if a correlation exists between plasma collected for clinical use and aged whole blood that was treated similarly to post-mortem samples. Two healthy male volunteer subjects with a history of opiate abuse provided informed consent and participated in this study. On separate occasions, each subject received a 60 and a 120 mg dose of codeine phosphate by the intramuscular route. Duplicate blood samples were collected at specified times following drug administration. Heparinized plasma samples were collected immediately from one set of samples. The second set of samples was processed as aged whole blood (without additives) by cycling three times between freezing, thawing and standing at room temperature overnight. After processing, all samples were frozen at -30°C until analysis. Samples were analyzed by gas chromatography/mass spectrometry following solid phase extraction (Clean Screen® columns) and derivatization (BSTFA with 1% TMCS). Only codeine was detected. Codeine concentrations in aged whole blood co-varied with plasma concentrations and were only slightly lower. The area-under-the-curve estimates (0-24 hrs) for aged whole blood averaged 86.9% of those obtained for plasma. Correlation coefficients (r) between the two types of samples were >0.98 and were highly significant ($p < 0.01$). These data suggest that deterioration of blood samples does not result in substantial loss of codeine and that concentrations in aged blood samples can be interpreted as being similar to those in freshly collected samples.

Keywords: Codeine, Plasma, Aged Whole Blood.

10

Detection of PCP in Sweat Collected using PharmChek™

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A procedure for the screening and quantitative confirmation of PCP collected from sweat using the PharmChek™ sweat patch has been developed. After soaking the absorptive pad from the patch in a methanol and buffer mixture to solubilize the drugs, the eluate is tested using a microtiter well ELISA manufactured by STC Diagnostics. Confirmation of presumptive positive samples is performed by GC/MS analysis following liquid/liquid extraction of an aliquot of that same eluate. A level of 25 ng/patch was adopted as the cutoff for both the screening assay and the confirmation assay.

Urine samples, collected at patch application and at patch removal, were obtained from known PCP users paid to wear PharmChek patches. In fifteen experiments, individuals tested positive by both patch and on one or both of the paired urine samples using a urine cutoff of 25 ng/ml. In the other four experiments, individuals could be considered positive in both urine and patch only if lower cutoffs were applied to one of the two specimen types. Multiple patches worn concurrently by some of the study subjects were tested to assess within subject variability.

Key Words: PCP, PharmChek, Sweat.

11

Ion Trap GC/MS/MS for Analysis of THC from Sweat

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THC concentrations in PharmChek sweat patch eluate were determined using a method based on ion trap GC/MS/MS. THC and a deuterated internal standard were extracted by a solid phase procedure, derivatized using TFAA and then back extracted. The technique of MS/MS involves isolation of a selected target ion from the EI spectrum. The target ion undergoes collision induced dissociation (CID) causing the formation of product ions. These product ions are then resolved into a full scan searchable spectrum. The formation of a searchable spectrum of product ions enables a degree of structural confirmation and sensitivity which cannot be achieved by EI alone. The ion trap GC/MS/MS method in this study used resonant ejection of all unwanted ions and RF field modulation during CID excitation. Results demonstrated linearity and detectivity for THC. Detection limits of 1 ng/patch comfortably met the requirements for confirmation of THC from PharmChek sweat patches.

Key Words: GC/MS/MS, THC, Sweat.

12

Ethyl Glucuronide - A Non-Volatile Ethanol Metabolite in Human Hair

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The advantage of hair analysis for abused drugs and their metabolites results from a longer detection window when compared with other specimens. However, detection of ethanol in hair is not so practical because ethanol is volatile and is lost to the environment upon detection.

We Investigated hair strands for the presence of ethyl glucuronide, a compound we have synthesized and characterized as previously reported (JAT, accepted for publication). Ethyl glucuronide is a minor ethanol metabolite whose fate in humans has not been sufficiently examined. Its role as an indicator of regular ethanol consumption at present is under investigation.

The water soluble ethyl glucuronide was found to be bound to hair fibres. By GC/MS analysis with different derivatization procedures (acetylation or silylation), we identified ethyl glucuronide in hair of persons with previous ethanol consumption, but not in abstinent infants.

Key Words: Ethyl Glucuronide, Human Hair, GC/MS.

13

Acceptance of Hair Drug Testing Evidence

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The admissibility of the results of analyses of blood, urine, hair, sweat, and other specimens is decided by judges or other hearing officers. They are guided by: (1) expert testimony, (2) scientific and legal writings, and (3) judicial decisions or case law.

(1) Relevant expert testimony on hair drug testing has been accepted, which demonstrates that it complies with: the Executive order mandating the use of the best available technology, the intent of the Federal workplace drug testing programs of identifying regular and occasional users, and the relevant parts of the Mandatory Guidelines.

(2) There are more than 200 scientific and legal writings, and about sixty 1994 abstracts as of June, 1994, on hair drug testing.

(3) Judicial decisions or case law have been documented in eleven criminal cases, six civil cases, and two administrative hearings. Five were workplace hair drug testing cases.

A most important judicial decision was made in a criminal probation revocation hearing by a judge who had participated in the SOFT Conference. He admitted the RIA hair drug testing evidence. US v Medina 749 F. Supp 59 (E.D.N.Y.1990) Criminal Law 304(1), 388(2) Fed. Rules Evid., Rule 403, 28 U.S.C.A.

Hair drug testing has been accepted by the courts and in other adversarial hearings.

Key Words: Hair Analysis, Case Law, Court Acceptance.

14

Diffusion of Opiates in Human Hair

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Diffusion experiments were performed using clipped hair fibers as diffusion bridges. The test series was conducted in a chamber with 100% humidity. One end of the strand of natural hair was put into an aqueous solution containing morphine, codeine and dihydrocodeine. The other end of the hair was dipped in a test tube filled with water. The concentration of the starting solution was 1 mg/mL. At fixed time intervals samples were removed, prepared and analyzed by GC/MS. Immediately after the water in the test tube became positive, the middle segment of the hair strand was clipped, washed and analyzed by standard procedures. Analysis of the formerly drug-negative hair sample always produced positive test results.

When pre-damaged hair-fibers, such as bleached hair, were used the diffusion increased dramatically. When any single hair fiber was coated with nail polish prior to incubation these effects did not occur in the hair over a 6 week period. The results clearly demonstrate that radial and axial diffusion of drug substances in human hair fiber is possible in the presence of water. Swelling of the hair fiber with radial diffusion is the first and main process to appear when hair is exposed to water. As soon as enough water molecules are present in the cross-sectional area of the hair fiber, the axial diffusion processes start. Only when the hair fiber is prevented from swelling does this effect fail to appear or appears very slowly.

Key Words: Hair Analysis, Opiates, Diffusion.

15

Stability of Cocaine and Metabolites in Human Hair Under Various Storage Conditions

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One of the potential promises underlying the advancing discipline of hair testing for drugs is that such compounds demonstrate incorporation stability. This promise states that once drugs/metabolites are incorporated into a specific region of hair, the incorporated materials remain at this site without significant degradation or movement. This potential is exciting, because segmented hair tests allow reconstructing the drug-use history of a subject.

While there have been studies of the spatial distribution of drug incorporation - for methamphetamine [Niwaguchi et al., 1983], haloperidol [Uematsu et al., 1989 and Matsuno et al., 1990], PCP, cocaine and marijuana [Baumgartner et al., 1989], morphine and codeine [Cone, 1990], nicotine [Kintz, 1992] and meprobamate [Kintz et al., 1993] - there is a working presumption imbedded in these experiments that the drugs, once incorporated, are stable to environmental "weathering" effects. If, in fact, this presumption is not correct, then segmented hair analysis to evaluate temporal drug use patterns should not be attempted.

Anecdotal evidence exists to suggest that cocaine is relatively stable in hair. Several groups have published findings for cocaine in hair from mummies [Moeller et al., 1992, Springfield et al., 1993]. However, there have been no published stability studies of cocaine in hair. In the present work, human hair from forensic casework which had been previously demonstrated to contain cocaine (some with metabolites as well) by GC/MS was washed, powdered and mixed to form a pool. 25-Milligram aliquots of this pooled positive hair were created and stored without specific light protection at either room temperature (22° to 26° C), under refrigeration (1° to 5° C) or frozen (-20° to -12° C) conditions. These aliquots were periodically tested using GC/MS beginning in November, 1993. Over the first 7 months (n=12 to 20 determinations per group), no differences have been observed for cocaine or cocaethylene in the three storage conditions (pooled t-test, P=0.01), and no trends are evident. However, preliminary data suggest that benzoylecgonine in hair may be sensitive to storage temperature. Among other utility, this study addresses the weight that should be ascribed to quantitative results in hair segment comparisons.

Key Words: Hair Testing, Cocaine, Stability.

16

Hair Testing for Cannabis in Spain and France: Is There a Difference in Consumption?

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In the present paper we compare the methods used in Sevilla (Spain) and in Strasbourg (France) for the analysis of cannabinoids (THC and carboxy-THC) in human hair. The methods are as follows:

Sevilla procedure: Hydrolysis with 11.8 N KOH (in the presence of THC-d_3 and THC-COOH-d_3) for 10 min at room temperature after washing the hair samples with methylene chloride at 37°C for 15 min, followed by addition of maleic acid, extraction with n-hexane/ethyl acetate and derivatization with heptafluorobutyric anhydride and hexafluoroisopropanol. Concentrations in the Spanish population ranged from 0.06 to 7.63, and 0.06 to 3.87 ng/mg for THC and carboxy-THC, respectively.

Strasbourg procedure: Samples were decontaminated twice with methylene chloride, then pulverized and dissolved in 1 N NaOH in the presence of THC-d_3 and THC-COOH-d_3. After cooling, drugs were extracted with n-hexane/ethyl acetate after addition of acetic acid and clean-up with NaOH and HCl. Derivatization with PFPA/PFP-OH was followed by GC/MS analysis. Concentrations in the French population ranged from 0.26 to 2.17, and 0.07 to 0.33 ng/mg for THC and carboxy-THC, respectively.

In order to validate both procedures, ten real samples were analyzed by the two laboratories. Results were in accordance within a 30% range. These data clearly demonstrated differences in cannabis concentrations in the hair of the two populations.

The usefulness of this paper was not only to compare both extraction procedures for cannabinoids in hair, but also to assess any possible differences in cannabinoid consumption between the Spanish and the French populations.

Key Words: Cannabinoids, Hair, Comparison Methods.

17

Possible Ethnic Bias in Hair Testing for Cocaine

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Cocaine binds to hair as a result of active drug use and from environmental exposure. The nature of this binding has not been defined but is likely to be related to the type, color, texture and condition of hair. There have been reports that some chemicals bind to Africoid and Mongoloid hair to a greater extent than Caucasoid hair. For drugs of abuse, recent reports indicate that drug incorporation may be greater in Africoid hair than Caucasoid hair. We investigated the nature and extent of binding of [^{3}H](-)cocaine to female Africoid and light-colored, female Caucasoid hair. Hair was homogenized in Tris buffer in a cell disrupter with borosilicate beads which provided a suspension of 1.0 mg hair/mL. Total binding to lipid and protein was determined by adding 100 nM [^{3}H](-) cocaine to suspensions. Specific binding to protein was defined as the fraction of [^{3}H](-) cocaine that was displaced by 10 uM (-)cocaine. Bound drug was isolated on filter paper with a cell harvester, and radioactivity was measured with a scintillation counter. Mean radioactive measurements (disintegrations per minute) of specific binding were as follows: Caucasoid (N = 8), 592 ± 488; Africoid (N = 9), 11,080 ± 8,225. Cocaine binding in the two groups differed significantly ($p < 0.01$). These data indicated that specific binding of cocaine to Africoid hair exceeded specific binding for light colored Caucasoid hair by approximately 10 fold. These preliminary results strongly suggest a potential for bias in hair testing for cocaine due to a greater number of binding sites in darkly pigmented hair. Consequently, Africoid and Mongoloid populations may be more likely than Caucasoid to produce a positive test result following cocaine exposure.

Key Words: Cocaine, Drug Binding, Ethnicity.

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Stability of Opiates in the Hair Fiber after Exposure to Cosmetic Treatment and UV Radiation

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The stability of opiates in clipped natural human hair was evaluated. Hair fibers were incubated with standard solutions of morphine, codeine and dihydrocodeine (pH 7.4). The starting concentration was determined prior to exposure. A commercially available bleaching, as well as perming formulation (Poly Blond Ultra®, Poly Lock®, Henkel, Düsseldorf, Germany) was applied to the hair strands according to the manufacturers instructions. Portions of the hair specimen were exposed to UV radiation. Two series were run, the first one at 60-80% humidity and the second one at 100% humidity. The middle segment of the hair strands was clipped, processed and analyzed with standard procedures. There was a decrease of about 25% in opiate content after exposure to UV, especially at 100% humidity when hair swelling was possible. After cosmetic treatment opiate concentrations declined dramatically.

The results demonstrated that environmental conditions and cosmetic treatments may influence the opiate concentration. This parallels the cuticle cells becoming irregular, partly broken, and lifted from the fiber surface. Severe bleaching results in breakdown of melanin granula, producing holes in the fiber. The ultrastructural changes of the hair fiber showed a correlation with a reduced rate of drug detection.

Key Words: Hair Analysis, Opiates, Environmental Stability.

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Drug Screening of Hair by Electrospray Ionization LC/MS

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The development of a rapid qualitative and quantitative mass spectrometric method for the analysis of drugs in hair is needed to maximize sample throughput in busy forensic laboratories with limited personnel resources. Chromatographic separation of hair extracts by high performance liquid chromatography (HPLC) with detection by electrospray ionization mass spectrometry (ESI MS) minimizes the need for sample clean-up procedures and does not require sample derivatization. ESI LC/MS gives abundant protonated molecular ions in the normal mode and can be operated in the collision induced dissociation mode for electron impact-like confirmational spectra. The high sensitivity of this technique permits the use of small samples.

The ESI LC/MS approach is being used for the screening of autopsy hair samples to determine drugs such as cocaine and opiates. The hair is extracted in solvent and filtered through 0.2 micron HPLC filters. The filtrate is evaporated to dryness and reconstituted in 25 microliters of acetonitrile for injection onto the LC/MS. Cocaine and its metabolites, benzoylecgonine and cocaethylene, have been determined in the hair samples. In addition, heroin users can be distinguished from users of other opiates by the presence of heroin and/or 6-monoacetylmorphine in the hair.

Key Words: Drug Screening, Hair, LC/MS.

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The Analysis of Cathinone in Urine Specimens by Gas Chromatography/Mass Spectrometry

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Khat is a shrub which grows in the highlands of East Africa and the Middle East and is chewed recreationally by the inhabitants of these areas. The psychoactive component of the plant is cathinone, a sympathomimetic amine. Recent military activities of American troops in these areas precipitated a request to establish a method to detect cathinone use in urine specimens. In man, cathinone is extensively metabolized; the keto group is stereospecifically reduced to norephedrine, which is the major urinary metabolite. Small amounts of parent drug are detected in the urine. The analytical method developed used 2 mL urine and 2 µg methylphenidate as the internal standard. The urine was alkalinized with 2 drops of ammonium hydroxide and extracted with 10 mL chlorobutane. The organic layer was evaporated to dryness after the addition of 0.1 mL 1% hydrochloric acid in methanol. The residue was reconstituted in 0.1 mL of 0.1% pyridine in heptane and 0.025 mL chlorodiflouroacetic anhydride and heated at 70°C for 30 minutes. After cooling, the solution was evaporated to dryness, reconstituted in 0.05 mL methanol and chromatographed on a DB-5 column with a split (30:1) injector. The oven temperature began at 150°C for 1 minute, increased at 30°C per minute to 260°C, holding for 5 minutes. Seven ions were monitored: m/z = 156,158, and 246 for norephedrine, m/z = 105,77, and 51 for cathinone, and m/z = 196 for the internal standard. Cathinone was well resolved from norephedrine and other commonly encountered sympathomimetic amines. For each compound, the limit of detection was 25 ng/mL, the limit of quantitation was 50 ng/mL and the linear range was 50-10,000 ng/mL.

Key Words: Cathinone, Norephedrine, Gas Chromatography/Mass Spectrometry.

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A Comparison of Immunochemical Urinary Screening Methods for Some Low Dose Benzodiazepines

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The Emit d.a.u.™, Abbott TDx™ and Abuscreen OnLine™ benzodiazepine assays, when used as intended by the manufacturers, are unreliable for the detection of intake of low doses of benzodiazepines. Reproducibility is higher and the cutoff intended by the manufacturers is lower with the respective serum assays. Therefore, we used the TDx and Emit serum assays instead of urine assays for urine screening. In addition, we determined the limit of detection (LD) for each assay and used it as a cutoff definition. Our aim was a comparison of these modified assays with low doses of benzodiazepines for curve stability, quantitative precision, cutoff reduction, cross-reactivity and the possibility for semiquantitative test result interpretation.

For calibration, Abbott Benzodiazepine™ urine calibration sets were used for all four assays. This was to guarantee the comparability of the applied assays. All assays were analysed with urine calibrators and urine samples regardless of whether assays were offered by the company as serum or urine assays.

Within-run precision showed coefficients of variation ≤ 3% in all assays. Within-day precision varied between assays. Emit's assays were less satisfactory when compared with the products of Roche and Abbott. The EMIT d.a.u. assay had the highest LD (least sensitive). An interesting result was found for Abuscreen OnLine when looking at cross-reactivity of flunitrazepam. In Roche's product information, a cross-reactivity of 55% for flunitrazepam is stated, while our results showed not more than 14%. The Abbott TDx assay was the most sensitive for 7-amino- and flunitrazepam, but was relatively insensitive for bromazepam.

All assays had good within-run precision. Syva products had problems with the stability of calibration curves because within-day precision was lower than the other immunochemical assays. Lowering the cutoff increased the detection of low dose benzodiazepines. The best results were obtained with Roche OnLine. The usefulness of this assay was limited due to the very low cross-reactivity of flunitrazepam and 7-aminoflunitrazepam.

Key Words: Urine Drug Screening, Immunoassay, Benzodiazepines.

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Stability of 6-Monoacetylmorphine (MAM) in Urine

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Objectives: We have observed that commercial preparations of 6-MAM contain variable concentrations of morphine. The goal of this work was to determine if 6-MAM in urine undergoes deacetylation to form morphine under three types of storage conditions encountered in forensic urine drug testing: 1) storage at room temperature for 1 to 8 days; 2) refrigerated storage for up to 10 days; and 3) frozen storage for up to 1 year.

Methods: 6-MAM was spiked into certified negative urine at a concentration of 185 ng/mL. On day 1, we assayed the morphine content of this preparation, then prepared aliquots for storage under three conditions: room temperature, 4° C and -20° C. Using a GC-MS assay with selected ion monitoring for the PFAA derivative of morphine, we measured the morphine concentration of the aliquots at selected intervals.

Results: The initial morphine concentration was 2.2 ng/mL. At room temperature, the morphine concentration increased steadily each day and was 9.3 ng/mL after 8 days. Under refrigerated storage, the morphine concentration increased more slowly, reaching 4.9 ng/mL after 10 days. After 3 months, 5 months, and 12 months, respectively, in the freezer, the morphine concentration was 3.1 ng/mL, 3.7 ng/mL, and 4.2 ng/mL.

Conclusion: 6-MAM in urine is relatively labile at room temperature, undergoing appreciable deacetylation to morphine. 6-MAM is relatively stable under refrigerated and frozen storage conditions commonly used by forensic urine drug testing laboratories.

Key Words: 6-MAM, 6-Monoacetylmorphine, Stability.

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Solid Phase Extraction and Spectrophotometric Determination of Paraquat in Biological Fluids

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A rapid and sensitive method to quantify paraquat in serum or urine is critical for the diagnosis and prognosis of paraquat intoxication. Isolation of this herbicide from biological fluids plays a very important role before analysis.

In this study, a SPE method without organic solvent was developed. Two milliliters of serum/plasma sonicated with 8 mL of distilled water, or 10 mL of urine, were pretreated with Amberlite IRA-401 resin, and then applied to a self-packed mini-column containing 50 mg silica gel (Wakogel Q12, 100-200 mesh). After washing with 2 mL of distilled water, the column was eluted with 2 mL of 2N NaOH at a flow rate of about 0.4 mL/min using a vacuum manifold. The eluate was reduced with dithionite reagent and paraquat was quantified with zero- or second-derivative spectrophotometry.

The detection limits of this assay are 0.005-0.001 mg/L with conventional spectrophotometry and 0.005-0.001 mg/L with second-derivative for serum and urine, respectively. The method demonstrates high extraction efficiency (recovery > 95%) and good precision (C.V. < 5%). The full procedure can be completed within 30 minutes. It is applicable for clinical and forensic toxicological purposes.

Key Words: Paraquat, Solid Phase Extraction, Spectrophotometry.

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Analysis of Illicit Amphetamine Analogues by Gas Chromatography/Mass Spectrometry with Stable Isotope Labeled Internal Standards

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The amphetamine analogues methylenedioxyamphetamine (MDA), methylenedioxy-methamphetamine (MDMA) and methylenedioxyethyl-amphetamine (MDEA) have been found on the illicit market for years. Their use, along with LSD, has recently increased and is often associated with so-called "rave" parties. Although drugs, such as amphetamine and methamphetamine, have a variety of different deuterium labeled compounds which can be used for quantitative analysis, some illicit amphetamine analogues do not have, or only recently have deuterium labeled isotopomers been made available for the quantitative analysis of these compounds.

Methylenedioxypropylamphetamine (MDPA), deuterium labeled methamphetamine and deuterium labeled isotopomers of the illicit analogues were evaluated as internal standards for the quantitative analysis of MDA, MDMA and MDEA in urine samples. Quantitative values using each of the above candidate internal standards were compared to evaluate the quantitative accuracy and reliability of each in the analysis of the most commonly encountered illicit amphetamine analogues.

Predictably, the deuterium labeled isotopomers yielded the most accurate and precise quantitative results. Statistical comparison of results, using analysis of variance, indicated quantitative values based on the isotopomers were significantly ($p<0.05$) more precise than using other compounds. Thus, for assays where quantitative accuracy is important, these compounds yield the most accurate and precise results.

Key Words: Amphetamine Analogues, GC/MS, MDA, MDMA, MDEA.

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An Enzymatic Digestion and Solid-Phase Extraction Procedure for the Screening of Acidic, Neutral and Basic Drugs in Liver - A Single Column Procedure

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A recently developed single column solid phase extraction procedure for whole blood and urine was tested for the screening of acidic, neutral and basic drugs in liver. Application of homogenized tissue to the column without further work-up produced clogging of the column. Therefore, various digestion methods were investigated in relation to the following criteria: the degree of digestion, removal of endogenous interferences, extraction yield, and stability of the analytes. A high pH during digestion worked well for the first two criteria but resulted in decomposition of the various analytes. Liver homogenate (100 mg liver; 2 µg drug) was mixed with buffer (pH 6) and centrifuged. The supernatant was applied to a mixed-mode Bond Elut Certify column. Acidic, neutral and weakly basic drugs were eluted. The liver pellet was enzymatically digested and after pH adjustment, centrifuged. The supernatant was applied to the SPE column and finally the basic drugs were eluted. The two fractions were analyzed by GC-FID.

Acidic, neutral drugs (barbiturates, glutethimide, etc.) and basic drugs (methamphetamine, methadone, antidepressants, etc.) gave recoveries of >70%. Benzodiazepines eluted in both fractions; the total recovery being >70%. RSD's (n=4) were found to be <9%.

Reference: X.-H. Chen. Mixed-mode solid phase extraction for the screening of drugs in systematic toxicological analysis. Thesis, Groningen, 1993.

Key Words: Systematic Toxicological Analysis, Enzymatic Digestion, SPE, Tissue.

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Analysis of Volatile Organic Compounds Found in Inhalant Abuse

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Paints, glues, cleaning products, degreasing agents, fuels and other commercial products contain a wide variety of volatile organic compounds (VOC's). When used in confined spaces, exposure to concentrations significant enough to be able to produce intoxication or impairment is possible. Exposure to VOC's can occur through unintentional environmental contact or through deliberate abuse via inhalation. While the analysis of VOC's in forensic samples has primarily focused on ethanol, specifically in relation to driving under the influence (DUI) cases, the occurrence of other VOC's in forensic samples is not uncommon. Laboratories must be able to detect the presence of other VOC's, confirm their identity and quantify them when necessary.

Over thirty different solvents, including the compounds commonly detected during blood alcohol testing, were evaluated on two distinctly different stationary phases. Columns (30 meter x 0.53 mm ID Rtx-BAC1 and Rtx-BAC2) were used in combination with a variety of flow and temperature parameters to determine the optimal chromatographic conditions for specific separations.

Differences in the selective retention mechanism of these two stationary phases permitted most compounds to be completely resolved on at least one stationary phase. Identification and confirmation of VOC's in forensic specimens could then be achieved by comparing relative retention times and elution order.

Key Words: Volatiles, Gas Chromatography.

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An Intoplicine Fatality

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Intoplicine (RP 60475F), a benzopyrido-indole derivative, is a new antitumour agent which inhibits DNA topoisomerase I and II. Intoplicine displays *in vitro* selective cytotoxicity for solid tumors. *In vivo* the drug has demonstrated a broad spectrum of activity against several murine tumors. Because of these interesting properties, intoplicine was selected for clinical phase I evaluation. A 60 year old man with intestinal cancer and lung metastasis, already pretreated unsuccessfully with chemotherapy (5-fluorouracil and leucovorin), died 5 hours after beginning a intoplicine treatment. The patient received intoplicine as an intravenous infusion. *Ante- and Post-mortem* blood samples were collected and analyzed for their intoplicine content. The drug was extracted from whole blood with solid-phase C2 Bond Elut columns. After separation by reversed-phase HPLC, intoplicine was tentatively identified and quantified by UV-DAD detection. Preliminary results indicated higher blood levels than maximum concentrations currently measured after therapeutic administration of intoplicine.

Key Words: Intoplicine, HPLC/UV-DAD, Postmortem.

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Amphetamine as an Artifact of Methamphetamine during Periodate Degradation of Interfering Ephedrine, Pseudoephedrine and Phenylpropanolamine: An Improved Procedure for Accurate Quantitation of Amphetamines in Urine†

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During periodate degradation of interfering ephedrine, pseudoephedrine, and phenylpropanolamine in the extraction of methamphetamine from urine, it was observed that a small amount of methamphetamine was demethylated to amphetamine. While all three interfering phenylpropanolamines could be degraded by periodate at pH 5.2 and above, this periodate mediated transformation of methamphetamine to amphetamine was observed only at pH 9.1 and above. Therefore, to avoid this transformation, pH 6.2 was used for the oxidative degradation of phenylpropanolamines. The excess periodate was then reduced with thiosulfate or ascorbic acid prior to the extraction of methamphetamine using a basic pH.

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† The opinions contained in this publication are not to be construed as official or as reflecting the views of the Department of Navy or the Department of Defense.

Key Words: Methamphetamine, Periodate Degradation, Urine Drug Analysis.

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Cyanide Intoxication From (Cassava) Manihot Exculenta Based Meal

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Background: Cassava, which constitutes 70% of the calorific intake of individuals in the tropics, contains a cyanogenic glycoside, linamarin. We report an acute poisoning involving a family of four.

Case Reports: A 25 year old welder, (AB), a 23 year old house wife, (MB), with two female children (TB, RB) aged 8 and 6 years were referred to the Emergency Centre, Lagos University Teaching Hospital (LUTH). All patients were reported to have vomited and complained of abdominal pains 1 hour after a meal of cassava (GARRI). The patient (AB) was in a coma, with dilated and fixed pupils. The pulse rate was 120 per min, B.P 110/60 mm-Hg which dropped to 60/40 within 5 hrs, and the patient subsequently died during intensive therapy. Patient (MB) had dilated and fixed pupils, spasmodic muscular movements with labored breathing. She was placed on supportive therapy but died of cardiorespiratory arrest after 24 hrs. The two children died within 3 hrs. of admission.

Conclusion: The blood and urine cyanide levels in the four patients were as follows: AB, 1.65 and 0.97 mg/L; MB, 1.97 and 1.45 mg/L; TB, 2.75 and 1.16 mg/L; RB, 2.08 and 1.19 mg/L. The tissue levels of cyanide varied from 1.06 - 2.01 mg/g. Acute cyanide poisoning from cassava is prevalent in Africa due to cyanide containing glycoside, linamarin which is hydrolyzed by the enzyme linamarase to release HCN. This report suggests that proper fermentation/processing method is necessary to detoxify the cyanogenic glycoside in cassava before consumption.

Key Words: Cyanide, Cassava, Poisoning.

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Sertraline and Paroxetine Quantitation in Medical Examiner's Cases

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Sertraline (Zoloft®) and paroxetine (Paxil®) are members of a new class of 5-hydroxytryptamine antidepressants which includes fluoxetine (Prozac®). Case histories and toxicological findings from Medical Examiner's cases from 1992 through 1994 that included sertraline or paroxetine were examined.

Blood was extracted and screened by GC/MS analysis. Poor chromatography of the metabolites necessitated the use of HPLC for quantitation. A photodiode array detector was used for quantitation (220 nm) and to rule out any interference (205 nm - 290 nm).

Analysis was performed on a C18 Alltima column from Alltech with a mobile phase of pH 3, 0.08 M sodium phosphate buffer:acetonitrile (2400:1600). Samples were made alkaline with pH 9.5 ammonium chloride buffer and then extracted with hexane:isoamyl alcohol (99:1). The organic solvent was extracted with 0.5N H_2SO_4. The aqueous layer (0.5N H_2SO_4) was injected onto the HPLC column. This is a simple, fast method of analysis for all samples (blood, liver and urine).

Sertraline concentrations in blood ranged from 0.06 to 0.38 µg/ml, norsertraline 0.05 to 1.48 µg/ml and paroxetine 0.16 µg/ml (one case).

Key Words: Sertraline, Paroxetine, HPLC-PDA.

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Determination of Bromovalerylurea (BVU) in Bone Marrow as Measured by High Performance Liquid Chromatography/Mass Spectrometry (LC/MS)

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BVU is a potent chemical capable of causing acute toxicosis. It should be included when screening for sedatives and hypnotics in legal and clinical medicine. To our knowledge, BVU analysis in the bone marrow of a suicide has not been reported. LC/MS was employed to measure BVU in bone marrow.

Methods: 1) The extracted bone marrow was pulverized with a mill and filtered through a 149 μm mesh sieve. 2) 0.1N HCl and ethyl ether were added to 0.5 g of bone marrow powder. The ethyl ether phase was evaporated, dichloromethane was added and evaporated. Then 100 μl of ethanol was added. 3) The sample was measured using LC/MS in the thermospray mode.

Results: BVU concentration in bone marrow was 95.4 μg/g.

Conclusion: 1) The present LC/MS method enables us not only to improve the sensitivity but also to measure BVU without interferences. 2) This result confirms the applicability of this method for human tissues.

Key Words: Bromovalerylurea, Bone Marrow, LC/MS.

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Unexpected Volatility of Barbiturate Derivatives: An Extractive Alkylation Procedure for Barbiturates and Benzoylecgonine

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An extractive alkylation procedure for benzoylecgonine (JAT, 11, 110-112, 1987) was modified to include the most common barbiturates. During the development of this procedure it was found that the final evaporation must be performed under very gentle conditions, because the propyl derivatives are unusually volatile. Two urine standards (200 ng/mL) were evaporated at 45°C and at an air flow of 5 L/min; one for 10 min and the other for 30 min. The loss of the analytes in the 30 min tube compared to the 10 min tube ((area of 10 min tube - area of 30 min tube)/area of 10 min tube) was: butalbital, 97%; butabarbital, 96%; amobarbital, 93%; pentobarbital, 86%; secobarbital, 80%; cyclopentobarbital, 61%; phenobarbital, 39%; benzoylecgonine, 16%; d_3-benzoylecgonine, 19%. The volatility of the barbiturates seems to be in inverse proportion to their retention times on the DB-5 capillary column.

Key Words: GC/MS Confirmation, Barbiturates, Extractive Alkylation.

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Deaths Associated with Amphetamine (AMPH), 3,4-Methylenedioxy-methamphetamine (MDMA), 3,4-Methylenedioxyethamphetamine (MDEA) or 3,4-Methylenedioxyamphetamine (MDA) Abuse

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Since the 1980's, the recreational use of (semi-synthetic) amphetamine derivatives has increased considerably. Today, several means of misuse and abuse are evident. Recently, we have observed an increase in deaths associated with the use of these compounds. Since data on this type of toxicity are still limited, we report our experiences over the last three years with special reference to MDMA ('ecstasy'), MDEA and MDA, although amphetamine cases are also included, because it is often used in combination with these substances or sold as 'ecstasy'. Quantification of these compounds in whole blood samples was accomplished by gas chromatography with a nitrogen-phosphorus detector.

Details of 11 deaths associated with the use of amphetamine only (n=5), MDA (n=1), MDMA (n=2) and MDEA (n=3) are reported. In 9 of these cases the use of these substances was considered the cause of death. In one 19 year old male (MDMA, 0.26 mg/L) signs of pre-existing cardiovascular disease were found at autopsy, whereas in another case (amphetamine, 7.4 mg/L) serious multiple injuries might have contributed to death. Postmortem toxicological analysis revealed the following whole blood concentrations; amphetamine only (mean (SD)), 2.4 mg/L (2.5); MDA, 10.0 mg/L; MDMA, 0.3 mg/L (+ 0.4 mg/L AMPH); MDEA, case 1, 10.3 mg/L, case 2, 12.7 mg/L (+ 2.1 mg/L AMPH), case 3, 0.5 mg/L (+ 0.8 mg/L AMPH). As a comparison, in cases related to 'driving under the influence' legislation, the following whole blood concentrations were found (mean (SD, n)): amphetamine, 0.32 mg/L (0.17, n=13); MDMA 0.22 mg/L (0.14, n=7); MDEA, 0.29 mg/L (0.25, n=5).

Although, MDMA and MDEA are widely misrepresented by users and agencies as being relatively safe ("safer than alcohol"), our recent data suggest that the increasingly, widespread use of these compounds coincide with an increasing number of fatal intoxications.

Key Words: MDMA, MDEA, MDA.

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Diazepam, Diclofenac Identified as Major Constituents in Over the Counter Herb Pills

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The unexplained incidence of positive benzodiazepine results has been troublesome, for employers, employees, laboratories, and Medical Review Officers (MRO). The high frequency of this occurrence in urine drug testing in the workplace, has led to extensive investigations and litigation.

Urine specimens that tested positive for benzodiazepines by enzyme immunoassay, were subjected to enzymatic hydrolysis, organic solvent extraction, TMS derivatization, and GC/MS analysis.

The pattern of the benzodiazepine metabolites suggested diazepam intake by the employees, but no diazepam administration could be linked to these individuals through the typical MRO inquiries. However, all admitted self administration of Chinese herb pills, under the names of Cow's Head Pills, Miracle Herb Pills, or PotentSex Pills.

Analyses of pill samples obtained by mail-order or from Chinese health food stores were performed by GC/MS and diazepam and diclofenac were identified. The same formulation appeared in all brands of pills analyzed.

Based on the above evidence we conclude that the self administration of Chinese herbal pills sold under the above listed brand names and possibly other untested brands could lead to a positive benzodiazepine and/or diclofenac drug test.

Key Words: Diazepam, Diclofenac, Chinese Herb Pills.

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A Case of Fatal Poisoning by Propofol

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This report details a fatal poisoning involving the intravenous injection of propofol; 2,6-bis(l-methylethyl)phenol. A 33 year old female surgical nurse was found dead in her room. Near the deceased, 4 empty ampoules of the anesthetic drug propofol (Diprivan®) and a used syringe were found. Putrefaction was evident and death was estimated to be about 1 week prior to discovery of the body. In the dustbin 6 empty packages of midazolam ampoules (Dormicon®) were found. Autopsy findings revealed several recent injection sites in the arm. Toxicological analysis was performed on blood and liver tissue homogenates, following acid extraction by cyclohexane (pH 4.7), GC (FID detection), GC/MS, HPLC (gradient system, diode array detection) and TLC (2,6-dibromquinone-4-chlorimide). A search for alkaline drugs, including midazolam, was negative. However, propofol was identified at concentrations suggestive of an overdose.

Key Words: Propofol, Postmortem.

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Automated Extraction Using SPEC SPE Columns

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The extraction of drugs from urine samples is labor intensive and prone to specimen identification mix-up errors. For a laboratory processing more than 50 specimens per day an automated approach reduces technical time and minimizes errors - an important consideration in a forensic environment. We have developed a system using off the shelf equipment and disposable solid phase columns (SPE). The Hamilton Co., Micro 2200 system is a programmable XYZ pipetting instrument commonly utilized for pipetting samples and reagents into ELISA plates. Modifications including a pressure-flow controlled sealing head coupled to appropriately designed racks make the instrument adaptable to using SPE columns. Low bed volume SPEC columns available from Ansys Inc., Irvine CA., are manufactured in a variety of absorption phases and in 3 mL and 6 mL configurations. The SPEC columns require small wash and elution volumes ranging from 100 µl to 500 µl; thereby, giving considerable speed to extractions.

Current APL drug of abuse confirmation methods were applied using the same reagents except for reduced wash and elution volumes. Sample volumes for cocaine, amphetamines, opiates and PCP are 1.0 mL and for THC-COOH 3 mL. The necessary hydrolysis step for both cannabinoids and opiates is automated using a heat block mounted on the instrument.

Aliquots in labeled 16x50 mm tubes are centrifuged and placed into a rack. Each tube's bar code or label is recorded; creating absolute documented aliquot custody control. Using a parallel operation format, SPEC columns are activated, samples with buffer and internal standard are added and pushed through using controlled pressure and flow rates, columns are washed and the drug eluted into tubes. As the elution volumes are less than 500 µL, evaporation time is minimized.

For the 1.0 mL specimen utilized for cocaine, amphetamines and PCP, output is 40 specimens/hr. For THC-COOH, including hydrolysis, output is 27 specimens per hour. Preliminary studies using ambient temperature, alkaline hydrolysis increases THC-COOH output to 40/hr. While opiates require a glucuronidase hydrolysis step for at least 4 hours; after completion of the hydrolysis, then output is also 40 specimens/hr.

SPEC column type for THC-COOH is C-18, for cocaine, amphetamine and PCP, MP-1, and for opiates, MP-3. Column recovery varies from 70-90% dependent upon the analyte. Linearity tested with calibrators up to 10-20X greater than commonly utilized cutoffs is excellent with $r > 0.99$. Precision using pooled real samples is in the range of 3-8%. Depending upon analytical needs individual batch analyses or mixed analyses can be performed.

Key Words: Extraction, Automated, SPE.

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A Fatal Poisoning Involving α-Chloralose

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Our report describes a fatal intoxication involving α-chloralose, a rodenticide as well as an obsolete anesthetic and sedative.

A 67-year-old woman was found dead in her bed. An open package of a rodenticide next to a cup, both of which contained α-chloralose, were found in the kitchen. Analyses of body fluids were done by head-space gas chromatography using an electron capture detector. The concentrations of α-chloralose in blood, urine, bile and stomach contents were 410, 10400, 260, and 3300 µg/mL, respectively. According to preliminary results, trichloroethanol, a metabolite postulated for α-chloralose, seemed to be present only in minor concentrations in body fluids.

Key Words: α-Chloralose, Trichloroethanol, Concentration in Body Fluids, Fatality.

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The Importance of Toxicological Analysis in Suspected Sudden Infant Death Syndrome (SIDS) Cases

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In 1992 the Orange County Sheriff-Coroner Toxicology Laboratory analyzed 69 Coroner's cases involving infants up to one year of age. Fifty-one of these cases were submitted as suspected SIDS deaths. The remaining 18 had other causes of death, such as homicide, asphyxia, congenital defects, etc.

Our laboratory performs a full body toxicological examination on all suspected SIDS cases. It is a challenge for the forensic toxicologist to analyze SIDS cases because:

1. limited blood and tissue samples submitted for testing (3 to 10 ml is a typical blood sample collected by our pathologists);
2. low therapeutic concentrations for most of the drugs found in these cases; and
3. interpretation of drug concentrations is difficult due to the lack of published toxicological and pharmacological data in infants.

We noticed that in 70% of the cases submitted as suspected SIDS, the babies were sick with common colds or other upper respiratory infections prior to death. Seventy-five percent of these sick infants reportedly were receiving over the counter or prescription medications. In 30% of these cases we identified and/or quantitated multiple drugs, including dextromethorphan, chlorpheniramine, promethazine, carbinoxamine, brompheniramine, phenylpropanolamine, codeine, and morphine.

Toxicological data will be presented and compared to data provided by the Pediatric Toxicology Registry Committee established by the National Association of Medical Examiners in the United States. Our findings in suspected SIDS cases suggest that the medical examination of these cases should include a thorough toxicological analysis.

Key Words: Toxicology, SIDS, Drugs.

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Diphenhydramine Concentrations in Clinical and Fatal Intoxications with Aviomarin

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Aviomarin (dimenhydrinate), the diphenhydramine salt of 8-chlorotheophylline, is available without prescription in Poland. Dimenhydrinate overdose cases are rather common. The drug was taken by people, aged 13-27, with the intention of attaining hallucinations (20 cases) and for suicidal purpose (5 cases).

The diphenhydramine concentrations in serum and whole blood taken from patients at the time of admission to the Clinic and after 2, 4, 7, 10 and 24 hours as well as in different autopsy materials were determined by gas chromatography after liquid-liquid extraction. The analyte was extracted with ethyl acetate from 5% ammonia solutions (recovery 92% ± 3.8%). The drug was analysed using a Pye Unicam 4500 GC equipped with a FID (240° C) and a 3% OV-17 packed column (220° C). The limit of quantitation of the drug was 0.2 µg/mL.

The dimenhydrinate poisonings were evaluated with respect to ingested dose, the drug level in serum and whole blood and clinical symptoms. Diphenhydramine serum levels were present in a wide range (2.5-33.7 µg/mL) due to differences in ingested dose (250-5000 mg), time between ingestion and admission to hospital (4-24 hours). No correlation was found between the ingested dose of the drug (reported by patients) and the plasma level. The ratio of whole blood/serum diphenhydramine concentrations was found to be 0.8 ± 0.2. The result represents the mean of 60 determinations. The diphenhydramine post-mortem blood concentrations ranged from 2.1 to 12.7 µg/mL. In two cases in which the cause of death was drowning, two boys, aged 13 and 15, had apparently overdosed with Aviomarin.

Key Words: Intoxication, Dimenhydrinate, Drug Concentrations.

40

Solid Phase Extraction of Exogenous Compounds from Whole Blood

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A procedure for the isolation of drugs and metabolites from whole blood (including postmortem and other forensic blood samples) is presented with a detailed separation scheme. Other matrices such as urine, bile and plasma can also be analyzed by this procedure. Unique to the process is direct addition of the blood sample to the solid-phase cartridge, without any modification. The cartridge is a mixed phase type consisting of both hydrophobic and cation-exchange material; a specialized filter above the frit traps large particulate matter which might clog ordinary cartridges. After column preparation, sample loading, and selective washing, acidic and neutral compounds are eluted with a dichloromethane/alcohol mixture; basic compounds are eluted with alkaline organic solvent. These fractions are then separately analyzed by GC/MS. Recovery and selectivity for a wide variety of compounds, including therapeutic drugs, illicit agents, controlled substances as well as their metabolites at typically encountered therapeutic concentrations is possible with this procedure.

Key Words: Blood, Solid-Phase Extraction, GC/MS.

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An Autopsy Case of Disopyramide Poisoning and Determination of Disopyramide and its Major Metabolite Mono-Isopropyldisopyramide by GC/MS

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A 33 year-old man was found dead on the bed in his room. One hundred empty packages of disopyramide (Rythmodan®) were found. Disopyramide and its major metabolite mono-isopropyldisopyramide in autopsy materials were determined by GC/MS. The concentrations of disopyramide and mono-isopropyldisopyramide in various specimens are listed in the following table:

	Concentration	
Specimen	Disopyramide	Mono-Isopropyldidopyramide
Blood	68.3 μg/mL	37.0 μg/mL
Urine	89.0 μg/mL	98.8 μg/mL
Gastric Content	309.3 μg/g	64.8 μg/g
Duodenal Content	268.6 μg/g	162.6 μg/g
Heart	98.5 μg/g	71.9 μg/g
Lung	171.4 μg/g	41.5 μg/g
Liver	145.8 μg/g	141.8 μg/g
Kidney	296.4 μg/g	106.1 μg/g
Brain	14.7 μg/g	17.3 μg/g

These results suggest that he took a large dose of disopyramide; and therefore, the cause of death was considered to be acute cardiac insufficiency due to disopyramide poisoning.

Key Words: Disopyramide, Mono-Isopropyldisopyramide, Poisoning.

42

Opiate Extraction, Detection and Differentiation in Urine Following Acid/Heat Hydrolysis

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Extraction, detection and differentiation of codeine, dihydrocodeine, hydrocodone, hydromorphone, morphine and related metabolites in acid/heat hydrolyzed urine challenges the laboratory due to labor intensive procedures, sample matrix interference, and difficulties in differentiating opiates and obtaining high recoveries. A procedure with supporting products has been developed that reduces labor and matrix interference while providing good opiate recovery and differentiation.

A 3-mL urine sample was acid/heat hydrolyzed for 25 minutes at 125°C and 15 psi. The hydrolyzed urine was diluted and buffered (pH 4.0) prior to extraction. The opiates were extracted and concentrated using SPEC®•6ML•MP3 solid phase extraction microcolumns. The extract concentrates were analyzed using TOXI•LAB® LTD™ Opiate Thin Layer Chromatography.

All five opiates were differentiated and detected with a limit of detection of 500 ng/mL. Ten samples were processed in 90 minutes with good recovery and very little interference from the sample matrix.

Key Words: Opiates, Solid Phase Extraction (SPE), Thin Layer Chromatography (TLC).

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Preliminary Study on Drug Screening of Tissue Specimens

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A preliminary study was performed by mass spectrometry to design a lipophilic drug screening method for tissue specimens. Costello's method for serum and urine extraction with dichloromethane as a solvent was modified. The method included a salting-out step by saturating with sodium bicarbonate. Using a silica gel column, samples were extracted and then analyzed by mass spectrometry.

Principle tissue components include triglycerides, cholesterol derivatives, fatty acids and phospholipids. A wide range of drugs were found to coelute with fatty acids and phospholipids but could be separated from neutral lipids with silica gel separation. In this process, whole blood and muscle can be used for drug screening, as the polar lipids, such as fatty acids and phospholipids, are present in relatively low concentrations.

Analytical interferences were found to coextract with drugs. The silica gel column performance provided useful information for the required type of sample preparation.

Key Words: Drug Screening, Tissue Specimens, Lipids.

44

Mercury in Bones

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We report the mercury content of fresh and prehistoric animal bones (sheep/goats/cattle) which we analysed with atomic absorption spectrometry (AAS) using cold vapor technique. The aim of this study was to ascertain the mercury levels of animal bones found in distinct areas of the Iberian Peninsula partly contaminated with relatively high natural mercury concentrations.

The evolution of prehistoric animal production systems in the Iberian Peninsula is little understood. Particularly, the question of prehistoric transhumance is still open. It is assumed that mercury, which is highly concentrated in the soil of the Almadén region, was absorbed by animals grazing there.

With one exception, the mercury content of all the animal bones analysed, was within the normal range: < 30 ng/g to 1040 ng/g. However, the highest mercury levels were found in bone samples excavated in the Province of Ciudad Real, near the Almadén region.

Key Words: Mercury, Bones, Transhumance.

45

Physical Characterization and GC/MS Analysis of α-Benzyl-N-Methylphenethylamine (An Impurity of Illicit Methamphetamine Synthesis) in Urine

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Methamphetamine is a popular drug of abuse, readily synthesized in clandestine laboratories. Illicit synthesis results in various contaminants which may contribute to the pharmacological/toxicological effects of illicit methamphetamine. Few impurities have been studied in vivo and little is known of their pharmacology/toxicology.

One such impurity is α-benzyl-N-methylphenethylamine (BNMPA). We synthesized BNMPA and its anticipated metabolites: N-demethyl-α-benzylphenethylamine, diphenyl-2-propanone and diphenyl-2-propanol. The purity and structure of these compounds and their heptafluorobutyric anhydride (HFBA) derivatives were confirmed by melting point, GC/MS and nuclear magnetic resonance (NMR).

A GC/MS method to detect these compounds in urine, using liquid/liquid extraction and derivatization with HFBA was developed. Using a 12 m. x 0.2 mm(id) x 0.33 mm HP-1 capillary column, BNMPA and its proposed metabolites were well-resolved from other common phenethylamine drugs and HHS-FUDT required analytes.

The limit of detection for BNMPA and metabolites was 2.5 ng/ml; the limit of quantitation for the four compounds was 25 ng/ml. The calibration curves were generally linear from 25 to 500 ng/ml. Typical within run CV's (at the LOQ) ranged from 13% to 20% (n=8). Between run CV's over one month at 25 ng/ml were 9 to 28% and at 500 ng/ml were 2.6 to 3.9%.

The detection of BNMPA or its metabolites in urine samples may provide a marker of use of illicitly synthesized methamphetamine.

Key Words: α-Benzyl-N-methylphenethylamine, Methamphetamine, GC/MS Analysis.

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Analysis of Benzodiazepine Compounds by Off-Line SFE/SFC

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Supercritical fluid extraction (SFE) and off-line supercritical fluid chromatography (SFC) were applied to the analysis of benzodiazepine tranquilizers. Analyte samples were selected from the marketed tablets as: diazepam (Cercin); ethyl loflazepate (Meilax); flunitrazepam (Silece); lormetazepam (Loramet); nitrazepam (Benzarin); and triazolam (Halcion); which are the most commonly encountered benzodiazepines currently prescribed and possibly abused in Japan. Fourteen additional benzodiazepines were also analyzed with this SFE/SFC system. Solid-extraction from each tablet was tried using the instrument JASCO / SUPER 200.

SFE analysis: Pressure, 200 kg/cm^2 ; Temperature, 40° C; Extraction time, 30 min; Flow rate, 3 mL/min CO_2.

SFC analysis: Column, Super Pak SIL (150 mm x 4.6 mm in diameter); Flow rate, 3 mL/min CO_2 with 0.1 mL/min methanol. The pressure and temperature were the same as in SFE.

Results: Recovery of each benzodiazepine varied with polarity. For example, triazolam had a recovery > 70%, while the recovery of nitrazepam was < 30% based upon UV-absorbance. Based on the retention time of nitrazepam (10.58 min), the relative retention times of the other compounds were determined as follows: 8.13 (triazolam); 0.37 (diazepam); 0.41 (flunitrazepam); 0.42 (lormetazepam); 0.60 (ethyl loflazepate); and 1.00 (nitrazepam), respectively.

Key Words: Psychotropic Drug, Benzodiazepine Derivatives, Analysis by SFE/SFC.

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A Procedure for the Solid Phase Extraction of Benzodiazepines and Tricyclic Antidepressants

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The goal of the present work was the development of a procedure which could separate tricyclic-antidepressants (TCA's) from benzodiazepines. The developed procedure has the utility of isolating either or both classes of drug from a single sample. A 200 mg bed of CLEAN UP® CEC18B2Z was conditioned with 3 mL of methanol and then 3 mL of a mixture of 90% water, 10% acetonitrile, in which dibasic potassium phosphate (7 grams per liter) was dissolved. The sample, one to five mL of urine or plasma, was then passed through the column. The column was washed with 2 x 2 mL of 90% water, 10% acetonitrile and the benzodiazepines eluted with 2 x I ml of 40% acetonitrile, 30% water, and 30% methanol. The TCA's remained on the column during the benzodiazepine elution step. The TCA's are quantitatively eluted by 2 x I mL 99% methanol, 1% 0.1N HCl.

Analytes are quantitated by HPLC utilizing UV detection.

Analyte	Per Cent Recovered in Benzodiazepine Elution		Per Cent Recovered in TCA Elution	
Diazepam	87 %	n = 3	0 %	n = 3
Lorazepam	91 %	n = 3	0 %	n = 3
Oxazepam	86 %	n = 3	0 %	n = 3
Clonazepam	94 %	n = 3	0 %	n = 3
Amitriptyline	0 %	n = 3	96 %	n = 3
Nortriptyline	0 %	n = 3	94 %	n = 3
Imipramine	0 %	n = 3	97 %	n = 3
Doxepin	0 %	n = 3	91 %	n = 3

This method represents a facile purification/separation technique for TCA's and benzodiazepines.

Key Words: SPE/HPLC, Benzodiazepines, Tricyclic-antidepressants.

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Comparative Analysis of Street Heroin Samples: Chemical and Statistical Surveys Indicate Origin of Samples

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Samples taken from over 150 street heroin preparations have been examined by combination of a wide range of analytical techniques. We suggest a complete analytical sequence based on analysis of opiates, diluents and adulterants by gas chromatography/mass spectrometry (GC/MS), thin layer chromatography (TLC), derivative spectroscopy and Rx diffraction, using a sample amount as low as 10-50 mg. The analytical characteristics were considered in conjunction with hierarchical cluster analysis of results, and it was found possible in many instances to discriminate between samples of different origin.

Key Words: Heroin, Chemical and Hierarchical Analysis.

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Determination of Carbamazepine and its Metabolites in Hair of Epileptics

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In the context of a systematic study about structural effects on the incorporation of drugs into hair and with respect to a possible use in compliance analysis, the concentration of carbamazepine (CBA) and its metabolites, carbamazepine-10,11-epoxide (CEO) and acridine (ACR) were determined, and a hydroxylated dibenzazepine was qualitatively identified by GC/MS in the hair samples of 30 epileptics. Generally, the sample preparation was performed by washing with acetone and extraction by methanol in an ultrasonic bath. For some examples this work-up was compared with hair digestion with 1 M NaOH. Tetraphenylethylene was used as the internal standard. CBA was quantified via its gas chromatographic artifact dibenzazepine. Since CEO is not stable under the GC conditions, it was analyzed via its artifacts 9,10-dihydroacridine-10-carbaldehyde (m/z = 209) and acridine-10-carbaldehyde (m/e = 207).

The concentration ranges were from 8.2 to 489 ng/mg for CBA, < 2.0 to 45 ng/mg for CEO and 0.7 to 7.7 ng/mg for ACR. No significant correlation was found between these hair concentrations and the plasma levels (3.6 to 11.5 µg/mL) or the daily doses (300 to 2800 mg) of CBA. The extreme deviations cannot be explained by differences in colour and diameter of the hair, age and sex of the subjects and in previous hair treatment by the subjects. Therefore, the investigation of hair samples appears not to be a suitable method for carbamazepine compliance analysis.

Key Words: Carbamazepine, Carbamazepine Metabolites, Hair Analysis.

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Determination of Methamphetamine in the Hair of Korean Abusers by GC/MS

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A sensitive method for the determination of methamphetamine (MA) and amphetamine(AM) in hair was developed by gas chromatography/mass spectrometry using stable isotope-labeled internal standards, amphetamine-d_5 and methamphetamine-d_5.

Hair samples were washed with MeOH, incubated with MeOH (1% HCl) overnight at 37°C with stirring and then extracted using solid phase extraction with a vacuum manifold. The extracts obtained were trifluoroacetylated and analyzed by GC/MS.

The calibration curves of MA and AM were linear from 0.5 to 250 ng. The limit of detection was 0.1 ng/mg in hair and a cut-off level of 0.25 ng/mg for both analytes was used. Hair samples of 671 MA abusers were examined during March 1993 to May 1994. Of the specimens tested, 32.1% were positive for MA with a concentration range of 0.7 to 106.8 ng/mg. The ratio of MA versus AM was 1.8-75.1 in the samples studied.

Hair analysis for MA by GC/MS is an effective method for identifying long-term drug abusers.

Key Words: Methamphetamine, Amphetamine, Hair Analysis.

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Determination of PCP and Its Major Metabolites, PCHP and PPC, in Rat Hair After Administration of PCP

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Phencyclidine (PCP) is a potent hallucinogenic drug and is primarily metabolized to 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP) and 4-phenyl-4-piperidinocyclo-hexanol (PPC). If a simultaneous detection method for PCP and the metabolites in hair was developed, it would be very useful for confirmation of past PCP use.

We tried to develop the analytical method for detection of PCP and its metabolites in hair using an animal model. Pigmented hairy rats were administered PCP at 0.1 - 2 mg/kg i.p. once daily for 10 days. After 4 weeks, hair samples were collected and extracted with methanol-5N HCl or methanol-TFA. Following the purification with Bond Elut Certify, the extract was derivatized with BSA for GC/MS analysis. The selected ions were monitored at m/z 200, 242 and 243 for PCP; 172, 288 and 331 for TMS-PCHP; and 91, 200 and 331 for TMS-PPC.

PCP, PCHP and PPC were simultaneously detected in rat hair at a concentration greater than 0.5 mg/kg. It was suggested that hair would be a very useful specimen for confirmation of active past PCP use.

Key Words: PCP, Hair Analysis, GC/MS.

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The Detection and Quantitation of 11-Nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid in Hair Using Tandem Mass Spectrometry

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The quantitation of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCA) in hair is a difficult technique due to its low abundance (1-10 pg/10 mg hair). We will discuss the extraction and quantitation procedure for THCA used in our laboratory. Extraction procedure: 15-30 mg of intact hair is washed three times at 37° C for 30 minutes each with 0.1 M phosphate buffer. The washed hair is then spiked with a d_3-THCA internal standard and digested with 10N NaOH at 70° C for one hour. The digested hair, is then extracted with hexane/ethyl acetate (9:1) and then back extracted under acidic conditions with hexane/ethyl acetate. Derivatization is accomplished with hexafluoroisopropanol and heptafluorobutyric anhydride. The resulting solution is analyzed using a Finnigan TSQ 700 MS/MS linked to a Varian 3400 GC equipped with a DB-5 capillary column. The mass spectrometer is operated in the negative chemical ionization mode using ammonia as the reagent gas, argon as the collision gas and monitoring parent ions 670/673 (d_0/d_3) with daughter ions 492/495 (d_0/d_3) and 344/347 (d_0/d_3). A positive is reported for a specimen at 0.5 pg/10 mg hair.

Key Words: 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid, Hair, MS/MS.

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Detection of LSD and NorLSD in Rat Hair and Human Hair

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LSD rapidly disappears from a user's urine because a psychoactive dose is very small and the drug is rapidly metabolized. Since these characteristics impose technical restraints on the detection of LSD in urine and blood more than a day or two after ingestion, the ability to detect LSD or its metabolites in hair would be very useful for confirmation of past LSD use.

We have sought to develop an analytical method for detection of LSD and its metabolites in hair, using an animal model. Pigmented hairy rats were administered with LSD at 0.1, 0.5 and 2 mg/kg i.p. once daily for 10 days. Newly grown hair was collected 4 weeks after the first administration and extracted with methanol/5N HCl. After evaporation, the extract residues were partitioned between 0.1M NaOH and methylene chloride:toluene(v/v, 3:7), and the organic phase was concentrated and derivatized by treatment with BSTFA/TMCS. For GC/MS analysis, selected ions were monitored at m/z 395, 293 and 279 for TMS-LSD and 381, 279 and 254 for TMS-norLSD.

LSD was detected in rat hair even under the lowest dosage regimen (0.1 mg/kg). NorLSD was detected, but only at the 2 mg/kg dosage. The results from analysis of hair from a human user of LSD will also be presented.

Key Words: LSD, Hair Analysis, GC/MS.

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A Study of Drug Abuse in High Schools by Using a Questionnaire and Hair Testing with Abbott TDxFLx

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The objective of the present study was to estimate the level of drug abuse (if any) among pupils attending secondary schools in Crete. Some reports from studies previously performed in central and northern Greek cities showed the existence of such a problem. Those studies were based on a questionnaire. Our intent was to compare the information obtained from a questionnaire with hair testing results performed within the questioned group. In order to conduct our research, official permission from the Ministry of Education was obtained. The questionnaire included 90 questions including family and personal data, and questions regarding the knowledge about controlled substances and possible use of them. The study was performed anonymously. To conduct hair testing additional permission from the parents' unions of the selected children were obtained. Hair testing was performed with an Abbott TDxFLx instrument after hair sample preparation and extraction by a variety of procedures. The drug classes included opiates, cannabinoids, cocaine, amphetamines and benzodiazepines. Positive results were confirmed by HPLC and GC techniques. The comparison of the questionnaire and drug testing results showed no significant differences within each group studied. A notable percentage of marijuana users ranging from 1.5 to 4% between several groups was found.

Key Words: Drugs in High Schools, Hair, TDxFLx.

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Distinction Between Amphetamine-Like Over-the-Counter (OTC) Drug Use and Illegal Amphetamine/Methamphetamine Use by Hair Analysis

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In order to distinguish the legitimate use of amphetamine (AP) - like drugs from illegal use of AP and methamphetamine (MA), an analytical method for parent drugs and their metabolites in hair of rats and humans dosed with AP - like drugs was developed using gas chromatography/mass spectrometry (GC/MS).

MA, AP and seven AP - like drugs; deprenyl, benzphetamine, mefenorex, furfenorex, fenproporex, ethylamphetamine, and dimethylamphetamine, were intraperitoneally administered to rats at 5 mg/kg once daily for 10 days. After 4 weeks, hair samples were collected and extracted with methanol/5N HCl. Following the purification with Bond Elut Certify, the extract was derivatized with TFAA for GC/MS analysis.

The parent compounds and/or unique metabolites were detected in the rat hair. Our method was applied to the detection of metabolites in the hair of human subjects who had taken deprenyl or benzphetamine. The parent compounds and unique metabolites as well as MA and AP were detected in the human hair. It was concluded hair would be a very useful specimen for distinction between OTC drug use and illegal use regarding amphetamines.

Key Words: Amphetamines, Hair Analysis, GC/MS.

55A

Supercritical Fluid Extraction of Drugs of Abuse from Post-Mortem Fluids and Tissues

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Supercritical fluids have unique solvent properties which make them excellent candidates for selective elution of target analytes from solid phase extraction (SPE) sorbents as well as directly from endogenous matrices. Presently, we report the successes and failures encountered when applying supercritical fluid extraction (SFE) to the selective elution of diphenhydramine, cocaine, cocaethylene, carisoprodol and phenobarbital from SPE sorbents as well as directly from postmortem fluids and tissues. Abraham solvation parameters were found to be useful in the selection of polarity modifiers as co-solvents using supercritical carbon dioxide. Addition of each modifier on a mole fraction basis was preferred over a percent (v/v) basis, allowing for a more accurate prediction of co-solvent effects. In addition, the generation of aerosols when decompressing a supercritical fluid into a collection solvent required special handling procedures for the human specimens and all operations including the SFE process were carried out in a all stainless Steel Class 11 Type A vertical downflow laminar flow containment hood.

A complete SFE method for the rapid determination of drugs in biological specimens has been developed based on SFE extraction/enzyme immunoassay (SFE/EIA). The method involves the extraction of target drugs with supercritical carbon dioxide followed by radial partition enzyme immunoassay (RPEIA) with front surface fluorescence detection. The quantitative analysis of phenobarbital in human liver tissues (20 to 90 mg/L) by this method compares favorably with the conventional liquid-liquid extraction/gas chromatographic (LLE/GC) method currently in use with a significant reduction in analysis time and uses inexpensive, non-toxic carbon dioxide, eliminating the use of halogenated solvents. The kinetics of the extraction from model systems (spiked phenobarbital in chicken liver matrix) have been studied and are surprisingly very similar to actual case specimens allowing for rapid methods development. Quantitative recovery of phenobarbital is achieved within 30 minutes and shorter extraction times have been successfully employed with the use of internal standards employing SFE/GC-MS.

Key Words: Supercritical Fluid Extraction, Enzyme Immunoassay, Postmortem Specimens.

55B

Co-Ingestion of Lethal Doses of Amphetamines and Heroin: Life-Saving Antagonistic Effects

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A case of oral ingestion of huge doses of the amphetamine derivative, 3,4-methylenedioxyethamphetamine (MDEA), and heroin is reported. The subject, a 25 year old male, had orally taken 40 tablets containing about 40 grams of MDEA and 12 grams of heroin. At admission, 2 hours later, he had developed acute-onset confusion, became extremely agitated with increased muscle rigidity. He was also rapidly becoming exhausted with progressive tachypnoea. Toxicological screening (GC-MS-TLC) was performed on gastric lavage fluid, urine and blood. The presence of trace amounts of paracetamol, heroin and "amphetamines" in the gastric lavage fluid was found. Initial serum concentrations were as follows (ng/mL): MDEA, 1400; cocaine, 5; heroin, 115. Metabolites of these drugs, namely, benzoylecgonine, MDA, monoacetylmorphine and morphine were detected in trace amounts. Twenty four hours following intensive care treatment, the concentrations of cocaine, MDEA and heroin in blood had respectively decreased to (ng/mL): negative, 0.91, negative. Our hypothesis is that the patient survived thanks to the opposing pharmacological effects of MDEA and heroin.

Key Words: Antagonism, MDEA, Heroin.

55C

Concordance of the Calypte Urine Screen for HIV-1 and Western Blot Confirmation Among Booked Arrestees in Manhattan

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Background: Previous studies among both high and low seroprevalence populations have shown high concordance between urine EIA and licensed Western blot testing using paired urine and blood samples. Given equivalence for HIV detection, urine may become a more convenient and less risky way to epidemiologically monitor HIV spread.

Objective: 1) To document the practicality of testing anonymous urine specimens to monitor HIV prevalence among a high risk population--arrestees booked for crimes in Manhattan. 2) To document the concordance and accuracy of the Calypte screen for HIV in urine with a modified Western blot confirmation.

Methods: The Drug Use Forecasting (DUF) program conducts self-reported interviews and collects anonymous urine specimens from 350 booked arrestees per quarter. During two quarters all urines from DUF-Manhattan were tested with the Calypte screen for HIV. Doubly reactive samples were subsequently confirmed by modified Western blot (WB). Calypte negatives were not confirmed, as 99.9% are reported to be WB negative by the manufacture.

Results: It was practical and efficient to test DUF-Manhattan urine specimens (N=691) for HIV with the Calypte screen. Seventy-nine percent (n=544) of all specimens were Calypte negative and assumed as true HIV negatives. Among the Calypte doubly reactive specimens, 110 were WB positive, 24 were indeterminates, and 13 were negative. For the Calypte screen, sensitivity was 99.1 percent and specificity was nearly 98 percent (regardless of whether WB indeterminate cases were counted as positive or negative or excluded). The negative predictive value was 99.8 percent, and positive predictive value was 89 percent. Accuracy was 95 percent.

Interpretation: The Calypte screen for HIV-1 in urine has very high concordance, sensitivity, and specificity for HIV detection. This screen will likely become very valuable as an independent indicator of HIV in high risk populations and for monitoring HIV spread in high and low seroprevalence cities. The relatively large number of WB indeterminates (n=24) and negatives (n=13) may be due to other factors (detection of related immunological problems, detection of seroconverters, etc).

Key Words: HIV Prevalence, Urine Screen for HIV-1, Western Blot Confirmation.

55D

Terfenadine Fatal Poisoning: A Case Report

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Terfenadine (Seldane®-Merrell Dow) is a non-sedating selective H1 histamine receptor antagonist used as an antihistamine to treat allergies. Although terfenadine is considered to be a relatively safe drug, several cases of terfenadine overdoses have been reported. This is a case report involving the death of a 48 year old woman with a history of multiple allergies. No anatomical cause of death was found at autopsy. Toxicological analysis of the postmortem blood identified terfenadine, a non-sedating antihistamine, and its two major metabolites, as well as trace amounts of diphenhydramine and lidocaine. Blood concentrations were: terfenadine - 0.44 mg/L, terfenadine carboxylate - 3.07 mg/L, and azacyclonol - 0.89 mg/L. The medical examiner ruled the cause of death to be a selfingested overdose of terfenadine. This terfenadine concentration in blood appears to be the highest reported in the literature to date.

Key Words: Terfenadine, Azacyclonol, Fatality.

55E

Urinary GC/MS Confirmation of the Common Benzodiazepine Metabolites: Ability to Differentiate Drugs

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The purpose of this study is to differentiate by GC/MS analysis between specific benzodiazepines that share common urinary metabolites. This differentiation is based upon understanding of the biotransformation patterns of different benzodiazepines, and is supported by excretion studies.

Urine specimens were subjected to enzymatic hydrolysis followed by solid phase extraction, TMS derivatization and EI GC/MS separation and detection. Closely related benzodiazepines such as diazepam, nordiazepam, oxazepam and temazepam could be identified by the different patterns of common urinary metabolites. Each pattern was specific for the particular drug. For example, a combination of urinary metabolites consisting of oxazepam, 4'-hydroxynordiazepam and/or nordiazepam indicates ingestion of nordiazepam. Some other drugs, like prazepam and helazepam have the same metabolic pattern, but in addition they also yield their specific 3-hydroxy-metabolites. Oxazepam is excreted unchanged. Diazepam is characterized by four major metabolites: oxazepam, temazepam, 4'-hydroxynordiazepam and/or nordiazepam. Temazepam yields oxazepam and temazepam only. Comprehensive GC/MS benzodiazepine SIM screen should also include specific metabolites of alprazolam, triazolam, lorazepam, lormetazepam and flurazepam.

Key Words: Benzodiazepine Metabolites, GC/MS, Biotransformation Patterns.

56

Please Give The Court Your Expert Opinion on When Cocaine or Marijuana Were Last Used

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The prolonged presence of cocaine (COC), delta-9-tetrahydrocannabinol (THC) and their primary metabolites in postmortem samples of hospitalized trauma or overdose victims were detected by gas chromatography/ mass spectrometry. The interval of survival in four individuals ranged from at least nine hours to five days. The presence and measured concentrations of these normally short-lived drugs appear to be inconsistent with published eliminations rates in healthy volunteers.

In the first case, with 9 hours of hospitalized survival, COC, cocaethylene (CE) and benzoylecgonine (BE) were found in postmortem blood at 0.03, 0.01 and 1.4 mg/L, respectively. In the second case, with 16 hours survival, postmortem blood yielded COC, BE, THC and THC-COOH concentrations of 0.01, 0.17, 0.009 and 0.07 mg/L, respectively. Postmortem urine and antemortem serum quantitations were also documented. In the third case, with 25 hours survival, COC and BE were 0.07 mg/L and 6 mg/L in postmortem blood and 0.8 mg/kg and 2.7 mg/kg in brain, respectively. In the fourth case, despite five days survival, both COC and BE were detected in bile and BE (1.4 mg/kg) was found in brain.

We conclude that caution is warranted in estimating the interval since last cocaine or marijuana use based on their presence in postmortem samples, at least when life support measures have been taken.

Key Words: Survival Time, Postmortem Drug Concentrations.

57

Comparative Investigation of Drug Concentrations in Cerebrospinal Fluid, Vitreous Humor and Blood

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The concentrations of drugs and their metabolites in cerebrospinal fluid *(csf)*, vitreous humor *(vh)* and blood *(bl)* were determined in 118 fatal opiate cases and in 42 intoxications by various other drugs using RIA and HPLC/DAD. The results were statistically analyzed with respect to data from the case histories including survival time, route of administration (oral, injection or inhalation), medical treatment, and single dose or chronic abuse. The findings can be understood in the context of physiological barriers, formation and regeneration time of *csf* and *vh* as well as drug and metabolite structures and a possible postmortem exchange.

With few exceptions, the concentrations were generally less in *csf* and *vh* than in *bl*. The concentration ratios *csf/bl* and *vh/bl* were between 0.5 and 0.7 for free morphine and between 0.2 and 0.4 for conjugated morphine. In most cases of heroin overdose, conclusions from such concentration ratios were difficult because of effects of earlier injections. After single high doses in cases with other drugs, *csf/bl* and *vh/bl* were < 0.1 after a very fast death and ratios up to 0.9 were found after survival of several hours or after steady therapeutic ingestion.

The forensic interpretation of analytical results from *csf* and *vh* instead of blood should be carried out with caution.

Key Words: Cerebrospinal Fluid, Vitreous Humor, Free and Conjugated Morphine.

58

Forensic Actuality of Anabolic Steroids: Violence and Addiction

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Widespread androgenic-anabolic steroids (AAS) use has been hypothesized recently to induce profound psychological effects similar to alcohol and opioid dependence. Long-term, high-dose AAS use is now often seen in top sporting activities. AAS abusers typically administer 10-100 times the medically recommended dose, alone or in combination with other hormones (e.g. hGH). In humans, AAS abuse is often associated with indiscriminate, unprovoked aggression. In forensic cases, the reputation of AAS to produce violent behaviour has been used as a defense to explain and excuse the aggressiveness of the assailant. Therefore, forensic toxicologists should now be familiar with the detection of AAS and their metabolites in bio-fluids. Circumstances surrounding a murder committed in Switzerland, in which the defendant was supposedly under the influence of AAS, prompted us to develop a GC-MS SIM screening method in which direct and enzyme hydrolyzed lipophilic fractions from urine samples were combined and then derivatized with MSTFA and TMS-Iodide. Our experience in doping analysis has shown that detection time varies from a few days to several months depending on the AAS consumed. Testosterone/epitestosterone ratios can be used to extend this time period by a factor of 10 to 20. When the defense's claim of AAS intake was stated in this case, results from the suspect's recent urine samples couldn't be used, as the murder was committed too long before.

Key Words: Androgenic-Anabolic Steroids, GC/MS, Forensic Samples.

59

A Fatal Case of Self-Poisoning with Aldicarb (Temik®)

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Aldicarb is used as a nematicide and as an insecticide in the form of microgranules at the concentration of 100 g/kg. This super toxic carbamate [(methyl-2-methylthio-2-propylidene) N-methylcarbamate] was involved in a fatal self-poisoning case.

A 30 year old white male was found dead at home with Temik® found near the body. Immunochemical screening for benzodiazepines, barbiturates, tricyclic antidepressants, salicylates, opiates, cocaine and cannabinoids was negative. The blood alcohol concentration was 1.52 g/l and the urine alcohol concentration was 2.51 g/l.

Determination of Aldicarb was achieved as follows: after extraction with chloroform at pH 5.5, Aldicarb was identified in blood and urine by GC/MS and quantified by HPLC/DAD. HPLC analytical conditions were: NovaPack C18 column; mobile phase: methanol / tetrahydrofuran/phosphate buffer, 65/5/30. Carbofuran was used as an internal standard.

Large amounts of Aldicarb were found in blood (4.8 mg/l) and urine (9.7 mg/l). We concluded that death was due to ingestion of Aldicarb and alcohol.

Key Words: Suicide, Aldicarb, Alcohol.

60

False Positive Post-Mortem EMIT Drugs of Abuse Assays Due to Lactate Dehydrogenase and Lactate in Urine

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A case of multiple false-positives in a post-mortem drugs of abuse urine screen using EMIT due to the presence of lactate dehydrogenase (LDH) and lactic acid is described. The decedent was a 43 year old black female with a history of diabetes mellitus, hypertension, ethanol and intravenous drug use and prostitution, who died at home while sitting on a sofa talking with friends. She had not sought medical attention in the past ten months. Post-mortem examination revealed a hypertensive-type intracerebral hemorrhage, cirrhosis, ascites and previously undiagnosed idiopathic membranous glomerulonephritis. Post-mortem urine screen for drugs of abuse with EMIT was positive for amphetamine, barbiturates, benzodiazepines, cocaine metabolites, opiates and propoxyphene. Gas chromatography/mass spectroscopy (GC/MS) of blood and vitreous showed only ethanol. GC/MS and alkaline drug screen of urine revealed only ethanol and ibuprofen. Alkaline and acid drug screens of blood detected no drugs.

EMIT uses the change in optical absorbance caused by the reduction of NAD+ to NADH by the enzyme glucose-6-phosphate dehydrogenase to give semi-quantitative or qualitative data on the presence of various analytes in urine. The post-mortem urine lactate level in this patient was >120 mg/dl, and urine LDH was 807 U/L. We conclude that NAD+ was reduced to NADH by the oxidation of lactate to pyruvate by LDH, resulting in a false-positive reaction.

Key Words: False Positive EMIT Screen, Lactate, LDH.

Rx: ? denature protein
ultrafiltrate, ultracentrifuge

61

Hydrocarbons in the Blood of Victims Exposed to Combustion Gas

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The purpose of this study was to identify combustion gases related to carbon monoxide (CO) intoxication by detecting volatile hydrocarbons in the blood of victims.

The analysis was performed with headspace capillary GC/MS. The monitored compounds included the series of aliphatic and aromatic hydrocarbons from n-pentane to n-dodecane and from benzene to n-butylbenzene. In some house fire cases, styrene was detected as well as benzene and toluene. In typical cases, using kerosene and gasoline as accelerants, both aliphatics and aromatics were detected. Kerosene and gasoline were distinguishable by the different hydrocarbon components in the blood. In the blood of victims exposed to the exhaust gas of gasoline vehicles, aromatics from benzene to trimethylbenzenes were detected, although aliphatics were not found to be above the detection limits.

This analysis is useful for the scientific elucidation of the incident, while the examination of carboxyhaemoglobin levels estimates only the CO toxic level.

Key Words: Hydrocarbons, Blood, Carbon Monoxide.

By looking at
Ar vs aliph vs styrene/
may be able to
differentiate source of
hydrocarbon

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A Method for Testing Postmortem Samples for Inhalation Anesthetics

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Based on reports in the literature, homicides in which the perpetrator forced his victim to inhale anesthetics are rare. Five such deaths in which chloroform was used as the incapacitating agent were described by Gettler and Blum in 1930, Bonnichsen and Maehly in 1966, and McGee et al. in 1987. At the annual meeting for American Academy of Forensic Sciences in 1994, another seven cases were reported by different research groups. One reason for the low rate of such crimes could be the problems the police, forensic pathologist and toxicologist have in identifying the possibility of inhalation anesthetics being involved in a death.

A method was developed for detecting solvents or gases with an anesthetic effect and for identifying as well as quantifying these in postmortem samples. It is based on extraction by direct headspace, separation by gas chromatography in a capillary column with an apolar stationary phase, and detection by mass spectrometry with an ion trap run in full scan mode. The method, allows the detection of anesthetics with retention index values, between 300 and 1300. Since chloroform has a short half-life in the blood (about 1.5 h.) and often occurs in association with chloral hydrate, some interpretation problems as to the origin of the inhalant may arise. This ambiguity can, however, be settled by also testing the stomach contents for volatile organics.

Key Words: Inhalation Anesthetics, Postmortem Samples, GC/MS.

N_2O
Halothane
Iso flurane
enflurane

63

High Performance Liquid Chromatography in a Fatal Azide Intoxication

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High performance liquid chromatography (HPLC) was applied to the analysis of azide in post mortem specimens in a fatal case of azide intoxication. Initial routine drug screening of the blood based on the enzyme multiplied immunoassay technique, thin layer chromatography, HPLC with diode array detection, gas chromatography with mass spectrometric detection and head space gas chromatographic analysis was negative. However, pathological findings and findings at the scene led to the search for azide.

The azide determination was performed using pre-column derivatization with 3,5-dinitrobenzoyl chloride followed by chromatographic analysis on an Ultrasphere ODS 5-µm column (15 cm x 4.6 mm, i.d.) and isocratic elution with acetonitrile:water (1:1, by vol) at a flow rate of 1 mL/min. Detection was based on photodiode array detection.

Azide was present in all matrices in the following concentrations : blood, 262 µg/mL; stomach contents, 754 µg/mL; bile, 1283 µg/mL; liver, 14 µg/g; kidney, 205 µg/g; respectively, and was determined to be the cause of death. Methemoglobin and cyanide content in the blood were also elevated.

Key Words: Azide Intoxication, HPLC (DAD), Postmortem.

also 35% Met Hb

$[CN^-]$ = 8 µg/mL! metabolite of N_3^-

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Arsenic Content in Cremation Ash After Lethal Arsenic Poisoning

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After an acute and lethal poisoning of a 7 year old child the question arose, whether the grandparents, who suddenly died one after the other within a short time frame 3 years previously, had also been poisoned with arsenic.

To determine the cause of death the cinerary urns of both grandparents were exhumed.

For the investigation we obtained the cremation ash of the child, who definitely died by arsenic poisoning, both grandparents and of an arsenic free deceased individual. The urn content was examined for arsenic by AAS-MHD and ICP-OES after dissolving in concentrated HNO_3/HCl.

The following mean values were found:

Child:	19.3 mg/kg
Grandfather:	4.08 mg/kg
Grandmother:	2.56 mg/kg
Unknown "Arsenic Free":	2.19 mg/kg

Summary: Arsenic content in cremation ash up to 4 mg/kg does not indicate a lethal arsenic poisoning.

Key Words: Lethal Arsenic Intoxication, Cremation Ash, Arsenic Content.

65

Simultaneous Identification and Quantification of Eight Benzodiazepine Metabolites in Urine Using Gas Chromatography - Mass Spectroscopy

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The benzodiazepines are a class of hypnotic sedatives that are among the most frequently abused prescribed drugs. The benzodiazepines undergo minor to extensive biotransformation followed by conjugation with glucuronic acid prior to excretion by the kidney. Very little of the ingested compound is excreted unchanged in the urine. Currently, most clinical and reference laboratories do not confirm and identify which benzodiazepines are present in urine. A gas chromatography-mass spectroscopy (GC/MS) method was developed to simultaneously identify the following major metabolites of the most commonly prescribed benzodiazepines: desalkylflurazepam (360.3, 359.3, 361.3 m/z), nordiazepam (341.3, 342.3, 343.3 m/z), oxazepam (429.4, 430.4, 431.4 m/z), temazepam (343.3, 344.35, 345.3 m/z), lorazepam (429.4, 430.45, 431.4 m/z), alpha-hydroxy-ethylflurazepam (288.25, 389.35, 290.25 m/z), alpha-hydroxy-alprazolam (381.35, 383.35, 396.4 m/z), and alpha-hydroxy-triazolam (415.35, 417.35, 430.35 m/z). Two milliliters of urine were hydrolyzed using beta-glucuronidase to yield an unconjugated benzodiazepine metabolite. The benzodiazepine metabolites were extracted from the urine using solid-phase extraction (Bond Elut Certify). The dried extract was derivatized using bis(trimethylsilyl)trifluoroacetamide and injected into a Hewlett Packard 5890 gas chromatograph interfaced with a HP 5970 mass selective detector. Selective ion monitoring was used to quantify the compounds using a four point standard curve (200-1000 ng/mL). Deuterated internal standards for nordiazepam-D_5 (347.15, 348.2, 349.2), lorazepam-D_4 (434.4, 436.4), temazepam-D_5 (377.25, 350.25), oxazepam-D_5 (435.3, 318.15, 406.3), and alpha-hydroxy-alprazolam-D_5 (386.25, 388.2) that represent specific mono- and di-TMS derivatives of the benzodiazepine compounds were used. Separation of the derivatized benzodiazepine metabolites was accomplished using a 12 meter HP-1 column. The injector port temperature was 250°C, detector 290°C, initial temperature 160°C increasing 20°C/min to 200°C held for 6 min followed by 20°C /min to 295°C. All compounds were distinctly separated using the GC column between 7 and 16.5 minutes. Correlation coefficients (r) ranged from 0.995 to 1.000. Performance of the procedure including intra-run, inter-run, limits of quantification were between 50 and 100 ng/mL and the recovery ranged from 85-108% depending on the benzodiazepine metabolite. Additionally, the performance of a visual screening test, the Triage® Panel for Drugs, as compared to GC/MS confirmation for benzodiazepine metabolites had sensitivity and specificity of 95 and 98%, respectively.

Key Words: GC/MS, Benzodiazepines, Quantification.

no conversion of PSE
baseline resolution of phentermine

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Send for reprint; V out EP, PSE

Rapid GC/MS Confirmation of Amphetamine and Methamphetamine in Urine as Propylchloroformate Derivatives

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A GC/MS method is described for the confirmation of amphetamines in urine. Internal standard and base are added to 200 µL of urine in a conical tube. The amphetamines are vortex extracted into an organic solvent containing propylchloroformate. Carbamate derivatives are readily formed at room temperature. The hydrochloric acid byproduct is concurrently eliminated into the alkaline urine. After removing the aqueous phase, a portion of the organic is analyzed by GC/MS.

The derivatives are separated on a methylsilicone capillary column. Full scan mass spectra are acquired with a Finnigan MAT ITS40 ion trap mass spectrometer. Quantitations using either deuterated analogues of amphetamine and methamphetamine or N-propylamphetamine as internal standards work equally well. Unique mass spectra are obtained for amphetamine, methamphetamine, phentermine, ephedrine, phenylpropanolamine, propylhexadrine and phenethylamine as well as other amphetamine analogues such as MDA and MDMA. Chromatograms are free from urinary interferences and possess low mass spectral backgrounds.

Key Words: Amphetamines, GC/MS, Analysis.

0.2mL urine + IS + 50µl K_2CO_3

0.2mL extractant/dut = 0.15µl hex
0.05mL CH_2Cl_2

1µl propyl chloroformate

Can get double dut @ 100x [ ]↑ of propyl chloroformate

maybe switch to E. Coli for codeine

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Cannabinoid Concentrations Detected in Human Urine by GC/MS are Influenced by the Method of Hydrolysis Used

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Cannabinoid metabolites are excreted as glucuronide conjugates and therefore must be hydrolyzed for the analysis of the free compound. This report compares the concentrations of Δ^9-tetrahydrocannabinol (THC) and two metabolites extracted from human urine following hydrolysis of the glucuronide bonds. For enzymatic hydrolysis, ß-glucuronidase from H. pomatia (mollusk) or E. coli (bacteria) was added to 1 ml of buffered urine, followed by adjustment of the pH to optimize activity for each enzyme. The samples were incubated at 37° C overnight. Base hydrolysis was performed with 1 ml urine, 0.5 ml 2N NaOH and incubation for 15 minutes at 60° C. All hydrolyzed specimens, and a non-hydrolyzed set of control samples, were extracted with hexane:ethyl acetate (7:1) and derivatized with BSTFA + 1% TMCS. Analysis of the specimens was achieved with a HP 5872 gas chromatograph/mass spectrometer operated in the electron impact mode, using temperature and inlet pressure programming. Results are summarized below.

	Mean Concentration (ng/mL)			
n = 3	Control (C)	Base (OH)	Mollusk (M)	Bacteria (B)
THC	0.8	1.4	3.1	37.8 a,b
11-OH-THC	1.0	1.7	4.6	82.2 a,b
THCCOOH	61.2	59.3	75.1 a,b	65.4 c

a = C vs OH/M/B; b = P<0.01; M vs B, p<0.01; c = M vs B, p<0.05

These data demonstrate the species dependent nature of glucuronidase activity in hydrolyzing cannabinoid metabolites and also demonstrate that cannabinoid concentrations in human urine are higher than previously reported. Supported by NIDA Grants DA-05850 and DA-06643.

Key Words: Cannabinoids, ß-Glucuronidase, Gas Chromatography/Mass Spectrometer.

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ASSIST

Forensic Toxicological Analysis of Δ^9-Tetrahydrocannabinol (THC) in Human Solid Tissues

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A reliable, simple and sensitive method was devised to determine the levels of Δ^9-tetrahydrocannabinol (THC) in human solid tissues: liver, brain, lung, kidney, spleen, muscle and adipose tissue. THC was effectively extracted with acetonitrile and interfering compounds were removed by washing with a solution of sodium hydroxide and hydrochloric acid. THC was then derivatized by methylation and subjected to gas chromatography/mass spectrometry (GC/MS). Deuterated methyl-THC (THC-CD_3) was used as an internal standard. The calibration curves were linear in the concentration range from 1 ng/g to 100 ng/g and the lower limit of detection was 1 ng/g in all samples examined. The accuracy and precision of the method were evaluated in every tissue sample, at two different concentrations, 5 and 50 ng/sample. The coefficient of variation ranged from 3.4 to 11.6 %.

We used this method to identify THC in tissues from an autopsied individual, and the distribution of this drug in the tissues was measured. Based on the data obtained, the toxic influence of this drug to the victim at the time of the incident was estimated.

Key Words: Δ^9-Tetrahydrocannabinol, Human Solid Tissues, GC/MS.

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Pharmacokinetics of Ibogaine: Analytical Method, Animal-Human Comparisons, and the Identification of a Primary Metabolite

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Ibogaine (NIH 10567) is an indole alkaloid originally isolated from the African shrub, *Tabernanthe iboga.* The use of ibogaine for the treatment of drug dependence has been based on anecdotal reports from addict self help groups that it may decrease the signs of opiate withdrawal and reduce drug craving for extended time periods. Preclinical studies in animals have shown that ibogaine reduces morphine self-administration, ameliorates signs of opiate withdrawal, and decreases cocaine preference. We have developed a procedure for detecting and quantifying ibogaine in urine and blood samples from rats, primates and humans. We have detected ibogaine and a single principal metabolite in blood and urine specimens. We have identified the primary metabolite as 12-hydroxyibogamine (noribogaine), by full scan electron impact gas chromatography/mass spectrometry (GC/MS).

The analytical procedure involves a solvent extraction under basic conditions with D_3-ibogaine as an internal standard. The organic extract is evaporated to dryness, and the residue is reacted at room temperature with n-propyl iodide in the presence of trimethylanilinium hydroxide in dimethyl sulfoxide. After derivatization, the reaction mixture is made strongly basic and extracted into an organic solvent. The derivatized drugs are back extracted and washed to remove impurities before a final basic extraction, concentration and GC/MS analysis. Quantitation is based upon the ratio of the molecular ions: $m/z = 310$ for ibogaine, $m/z = 313$ for D_3-ibogaine, and $m/z = 338$ for noribogaine-propyl ether. The method is linear and gives acceptable reproducibility.

Pharmacokinetic studies compared across species suggest that the primary metabolite of ibogaine may have a very extended biological half life. Studies are underway to determine the relative contribution of parent drug and metabolite to the complex CNS pharmacological profile.

Key Words: Ibogaine, Metabolite Identification, Pharmacokinetics.

1mL sample + 5mL MeOH – vortex / centrifuge
evap to dryness – reconst w/ H_2O

Condition: MeOH/H_2O
load:

70

wash: H_2O / hexane / pentane
elute: 10% 1-butanol in DCM

LC: ODS

Analysis of Nifedipine in Whole Blood Samples

H_2O/ACN 1:1 + cetrimide
λ 238nm

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ion pairing reagent

Nifedipine is an anti-anginal/anti-hypertensive drug which acts as a slow calcium channel blocker. It is widely used in the UK and occurs regularly in medicolegal cases requiring toxicological analysis. Because of the inherent instability of the drug due to the presence of a dihydropyridine ring in its structure, its analysis requires careful sample handling, particularly the exclusion of light. Previously published methods, for clinical plasma samples, have used gas chromatography with electron capture detection. This paper describes a method for the analysis of the drug in whole blood, suitable for autopsy samples.

The isolation procedure uses solid phase extraction (SPE) on octylsilica following deproteination with methanol. Octylsilica was found to be the most suitable SPE material amongst the phases evaluated (octadecylsilica, ethylsilica, phenylsilica and cyclohexylsilica). HPLC was carried out on ODS Hypersil (5μ) using mobile phases based on either methanol/water or acetonitrile/water/cetrimide. Detection was by UV at 254 or 238, respectively, depending on the mobile phase. Nitrendipine was used as the internal standard.

Validation of the method indicated that nifedipine and its main metabolite, dehydronifedipine, had a detection limit of 5 ng/ml blood and that the method was linear over the concentration range of interest (normal therapeutic concentrations are below 200 ng/ml). The method was evaluated using case samples, spiked autopsy blood and whole blood clinical specimens.

Key Words: Nifedipine, Whole Blood, SPE/HPLC.

UTR: 17–100ng/mL

nitrendipine I.S

light, heat sensitive

GC = "may cause problems"

LLE: difficult to get high recovery / clean extracts

SPE: C8

Toxic 8.3μg/mL, 7μg/mL blood
(2 cases)

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Capillary Blood Collection by Paper for Graphite Furnace Atomic Absorption Spectrometry

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A new method for the screening of blood lead is described utilizing graphite furnace atomic absorption spectrometry (GFAAS) and capillary blood from a finger stick collected on Schleicher & Schuell 903 paper. The dried blood is eluted by treatment with 5% v/v nitric acid-0.75% v/v Triton X-100 to yield an aqueous solution for analysis, so that neither burning nor acid digestion is required. Calibration is performed with aqueous lead standards.

A parallel study of 100 samples of capillary blood obtained by a finger stick and corresponding venipuncture samples assayed by a matrix modifier method (Analyst 112:1701-1704 (1987)) gave a correlation r = 0.973. Additional advantages include 5 month stability under room temperature storage conditions, ease of transporting samples collected on paper, and the relatively more pleasant means of obtaining blood from young children by finger stick.

Thus, the proposed method offers a suitable alternative to the use of venipuncture blood for lead screening.

Key Words: Capillary Blood, Paper Collection, Graphite Furnace Atomic Absorption Spectrometry.

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Instrumentation for Direct and Reversed Extractive Dialysis

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Extractive dialysis is a clean, labor-saving method for extracting aqueous suspensions (and solutions), and was first described at the TIAFT meeting in 1979 (Forensic Toxicology, ed. J. S. Oliver, Croom Helm, London, 1980, pp. 104-108). The aqueous phase is sealed in a dialysis bag and placed in a bottle containing the organic solvent. Extraction and filtration proceed simultaneously. Type and volume of solvent, pH as well as a number of extraction steps can be selected just as in separatory funnel extraction.

We now present a simple tool which permits "Direct Extractive Dialysis", as well as "Reversed Extractive Dialysis" with small solvent volumes. It facilitates pH changes and other adjustments between the single extraction steps. Several membrane materials were compared, especially with respect to their possible adsorption of basic components. The advantages gained with a combination of enzymatic treatments and extraction are emphasized.

Key Words: Extraction by Dialysis Against Organic Solvents, Reversed Extractive Dialysis, Extraction of Suspensions.

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Microwave: A New Tool in Forensic Toxicology

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Microwave irradiation is used mostly as an alternative heating method for hotplate digestion for metal determination. We describe a method for the determination of selected drugs in human blood/serum using microwave extraction.

The extraction of organic substances requires special instrumentation. We performed the present extractions in an "open-vessel" system and under temperature controlled conditions. Before irradiation with microwaves, an adequate solvent mixture was added to the buffered specimen. Diazepam, nordiazepam, propoxyphene, norpropoxyphene, lidocaine and methadone were tested as model substances. The quantitation was performed by GC/NPD. The procedure has been applied to a number of forensic cases. The results have been compared with the results from liquid/liquid extraction. The concentrations of drugs determined after microwave extraction were significantly higher than those obtained by conventional liquid/liquid extraction.

Key Words: Extraction, Drugs, Microwave.

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Lamotrigine Analysis in Serum and Whole Blood by HPLC

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Lamotrigine is an anticonvulsant medication recently introduced to the North American market. It is chemically unrelated to any currently available anticonvulsant drug. Based on animal models, lamotrigine would have an antiepileptic profile useful in the treatment of partial seizures and tonic-clonic seizures. The objective of this study was to develop a quantitative HPLC assay for lamotrigine in serum for clinical studies and in whole blood for post-mortem analysis. Lamotrigine was extracted from serum/whole blood at alkaline pH into ethyl acetate after addition of the internal standard (BW725C78). After mixing, the organic layer was evaporated prior to dissolving the residue in methanol for HPLC analysis which was performed isocratically on an RP-8 column (5 µm) with a mobile phase of water/0.5 M phosphate buffer (pH 6.5)/acetonitrile (780/10/200). Calibration was performed with five serum/blood standards (2-32 µmol/L). The flow rate was 1.6 mL/minute and the eluant was monitored by UV absorbance at 306 nm. The procedure was linear from 2 to 250 µmol/L and recovery was 88% at 25 µmol. Within run precision was 1.9 and 0.8% C.V. at 14.1 and 31.1 µmol/L, respectively. Between run precision was 4.1 and 2.5% C.V. at 13.6 and 31.6 µmol/L, respectively. Lamotrigine measured in 20 serum samples ranged from 3-125 µmol/L. At room temperature, lamotrigine in serum was stable for at least 7 days. Interference studies were performed on several serum specimens containing other anticonvulsant drugs and on 20 random post-mortem blood specimens. The only potential interfering drug was carbamazepine. We conclude that this is a reliable method for quantitation of lamotrigine in serum and whole blood.

Key Words: Lamotrigine, HPLC, Quantitation.

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Qualitative and Quantitative Analysis of Drugs of Abuse in Clinical and Forensic Blood Samples by HPLC with Different Detectors

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In the Netherlands and in several other countries toxicological screening of blood/plasma samples is carried out by means of HPLC with diode array detection (DAD) and an on line library (STIP system). In the beginning this method was only suitable for drug concentrations in the mg/L range and with a limited number of different compounds. By using solid phase extraction (SPE Bond Elut Certify, Varian), HPLC (Hitachi L6200A) with gradient elution (from 100 % to 25 % phosphate buffer, pH = 3, with acetonitrile in 35 min.) on a Lichrospher 60 RP-select B 5 µm column; 125 x 4.0 mm ID column (Merck 50981) and with a newer more sensitive DAD with more diodes (Hitachi L4500) in series with a fluorescence detector (Hitachi F 1050; Sens 0.05; Ex 290 nm, Em 340 nm) and an electrochemical detector (Amor; 085 V/ox 1000 nA), we now have a fast and suitable method for the qualitative and quantitative determination of nearly a thousand prescribed drugs, toxic compounds, most drugs of abuse in the Netherlands and active metabolites in 1 mL of plasma, urine or forensic blood sample.

The mean time required for clean up, solid phase extraction, concentration, gradient elution, identification with the on line library and quantification is about 3 hours for a standard sample. The lower level of quantification (LLQ) with DAD is equal to or less than 0.1 mg/L, but with fluorescence detection and ECD (opiates) a much lower LLQ is found.

The system has been compared with GLC with mass selective detection (ion-trap detector). The HPLC gives suitable results in a shorter time, is cheaper and requires less training and experience than the GC/MS method.

Key Words: HPLC-DAD, DOA Testing, STA.

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Inhalant Abuse: A Dangerous Trend

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Inhalant abuse is a grave problem and has great national implications. A recent meeting of the International Association for Chemical Testing dedicated one full morning of a four day meeting to inhalant abuse.

Why do youngsters sniff, where do users find inhalants, do inhalants cause any harm, can we identify abusers, and who are the sniffers? This report answers these questions, and addresses some of the difficulties associated with attempts to find an answer to the inhalant abuse problem. The problem seems to cut across racial, economic, educational, and sexual lines, although it predominantly involves the young.

Samples were collected with Toxic Vapor Tubes, the trapped sample was then transferred to a headspace vial, and a parachlorofluorobenzene/methanol solution was added as an internal standard. Samples were analyzed using headspace/dual column gas chromatographic procedures. A volatile standard solution containing fourteen compounds, including acetone, toluene, and ethyl acetate, was analyzed using the same procedure. Urine and/or breath samples collected from the same subjects were analyzed and the results compared. Three case studies included: (a) a breath sample from a 33 year old male yielded toluene, and (b) a urine and breath sample from an eighteen year old male. The breath sample indicated the presence of toluene and the urine sample was negative, and (c) a breath and urine sample from an eighteen year old male produced a positive breath test for toluene and a negative urine test.

The dilemma is national, and we need a concerted national effort to attack the problem. Just as the user who will quit when they decide to do so, we can truly begin to understand inhalant abuse only when we admit it is a major problem.

Key Words: Inhalant Abuse, Sample Collection, Analyses.

Butane, Toluene, other ROI

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Analysis of 11-nor-Δ^9-Carboxy-Tetrahydrocannabinol, Morphine and Benzoylecgonine in Serum Samples Using OnLine® Kinetic Microparticle Immunoassays

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These experiments were designed to test whether commercially available urine kinetic microparticle immunoassays (KMIA's, OnLine®) could be used to detect abused drugs in serum samples. Transition of the assay from urine to serum offers two primary challenges. First, serum concentrations of many drugs are often lower than in the urine, and decreased limits of detection (LOD) may be needed. Second, the serum matrix may have an effect on the assay; greater inter-sample variation being a common concern. For these experiments, drug-free sera (or urine for comparative purposes) were fortified with either morphine, benzoylecgonine (BE) or 11-nor-Δ^9-carboxy-tetrahydrocannabinol (THC-COOH). We have compared the analysis of unextracted sera with extracted sera which has been reconstituted in a phosphate buffer (pH 7.4). With all 3 assays, we could analyze unextracted serum using the protocols the manufacturer established for urine analysis at the suggested NIDA/SAMSA cutoffs. Attempts to lower the LOD by increasing the sample volume analyzed were only partially successful, as slight increases in the sensitivity of the assay at the lower concentrations resulted in corresponding decreases in the dynamic range of the assay. When samples (1 mL) were extracted with 7 mL of chloroform:isopropanol (9:1), dried, and reconstituted with 0.5 mL buffer, the sensitivity of the low end increased without reducing the dynamic range for the opiate and BE assays. No enhancement was found using this approach with the THC-COOH assay. Analysis of 15 to 27 different serum samples resulted in large between-sample variation in the signals. For morphine at 100, BE at 100 and THC-COOH at 50 ng/mL, the CV's were 6.8, 7.3 and 6.5%, respectively, in unextracted samples. These were reduced to 4.3, 3.6 and 4.3%, respectively, in extracted sera. Serum samples can be analyzed by the OnLine KMIA technology developed for urine testing. Extraction of the sample with reconstitution in a smaller volume of buffer decreases the LOD of some of the assays, and reduces the between-sample variation for all 3 assays. The latter factor is essential for establishment of reliable cutoffs that are lower than those typically used for urine samples.

Key Words: KMIA, Serum, Matrix.

for extracts, reconstitute first w small amt R-wt, then buffer

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Colorado DRE (Drug Recognition Expert) Program Statistical Report, July 1992 - June 1994

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The Toxicology Unit of the Colorado Department of Health Laboratory Division has performed the analytical component of the Colorado DRE program since October of 1988. The primary sample is urine with delayed breath specimens often submitted for the inhalant category. All urine samples are subjected to a panel of testing using EMIT and TLC for the screening process and GC/MS for confirmation. Analyses for inhalants and LSD screening are performed only upon request. In this two year period our laboratory analyzed 1353 urine samples submitted by certified DRE's. At least one drug was identified in 85% of the samples. The majority (74%) of the time the DRE identified only one category in their opinion. Cannabis was the most common DRE opinion (56%), followed by CNS stimulants (17%), and CNS depressants (13%). Toxicology verification of the DRE category ranged from a low of 21.1% for the hallucinogens to a high of 82.6% for the cannabis category. Cocaine was the primary source for CNS stimulants, codeine for narcotic analgesics, and benzodiazepines for CNS depressants. The DRE opinion matched at least one drug admitted by the subject 97.6% of the time. Toxicological analysis matched at least one drug admitted 85.7% of the time and identified additional drugs in 187 (24.7%) instances. Polydrug use (excluding ethanol) was identified by toxicology in 287 (24%) of the samples. At least one DRE opinion category was correct in 97.2% of the polydrug instances.

Key Words: DRE, DUID, Urine Drug Testing.

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A Review of Khat as a Drug of Abuse: Urinary Excretion Profile in a Human Volunteer Study

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Catha edulis Forsk., an evergreen shrub grows wild and is also cultivated in parts of East Africa and the Middle East. It is more commonly known as "khat" and its leaves have been used for its CNS-stimulating and anorexic effects for over a century in this part of the world. S-Cathinone (S-α-aminopropiophenone), the dominating alkaloid in the fresh drug and other phenylalkylamines (S,S-norpseudoephedrine, R,S-norephedrine etc.) have been identified. The abuse potential of cathinone, the main psychoactive, amphetamine-like compound, was officially recognized by the UN in 1985. In the United States, khat and cathinone were scheduled as controlled substances in 1992.

A review of the stereochemistry of the khatamines will be presented. A controlled clinical study with standardized khat leaves in six human volunteers showed a significant correlation between amphetamine-like effects and plasma cathinone concentration. Peak plasma values of about 120 ng/mL of cathinone were observed between 90 and 150 minutes. Most of the drug and metabolites were cleared from plasma by the end of 8 to 9 hours. Urine concentrations showed a wide range of 0.37 to 1.28 ng/mL, 2.0 to 37 ng/mL and 7 to 113 ng/mL, respectively, for cathinone, R,S-norephedrine and R,R-norpseudoephedrine. The wide range of urinary concentrations of cathinone and the metabolites observed is mainly due to inter-individual differences in the mastication and absorption process. The data have significant implications in the context of drug-testing.

Key Words: Khat, Cathinone, Pharmacokinetics.

Cathinone - ingredient of khat

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Methcathinone - illicitly synthesized (CAT)

Multi-Drug Use Patterns of Drug Users in Baltimore/Washington D.C.

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Multi-drug use is presumed to be prevalent in drug users, but there have been few surveys on the extent or pattern of concurrent use of more than one illicit drug. We tested urine samples from a total of 633 recruitment volunteers who applied over the last 6 months for participation in research studies at the Addiction Research Center in Baltimore, MD. Urine samples were collected at first contact and tested by immunoassay (EMIT II) for amphetamines (Amps), barbiturates (Barbs), benzodiazepines (Benzos), cannabinoids (THC), cocaine (Coc), methadone (Meth), opiates (Op) and phencyclidine (PCP) at DHHS cutoffs with the exception of THC (50 ng/mL). Study protocol requirements influenced which drug user population was recruited. Patterns of multi-drug use (% of total) were as follows: negative, 21.6%; 1-drug, 30.5%; 2-drugs, 34.3%; 3-drugs, 11.8%; 4-drugs, 1.8%. Single drug occurrences were as follows (% of total 1-drug): Amps, 1.2%; Barbs, 0.4%; Benzos, 1.1%; THC, 9.2%; Coc, 60.3%; Meth, 1.5%; Op, 26.3%; PCP, 0%. Multi-drug combinations most prevalent were as follows (% of multi-drug set): 2-drugs, Coc/Op, 71.9%; Coc/THC, 8.8%; 3-drugs, Coc/Op/THC, 45.5%; Coc/Meth/Op, 13.4%; 4-drugs, Benzos/Coc/Meth/Op, 23.6%, Benzos/Coc/Op/THC, 10.7%. PCP was detected in urine of 7 subjects, all in combination with other drugs as follows: Benzos/PCP (N=1); Meth/THC/PCP (N=1); Coc/Op/PCP (N=1); Benzos/Op/THC/PCP (N=1); Coc/Meth/Op/PCP (N=1); Benzos/Coc/THC/PCP (N=2). These data demonstrate that multi-drug use is a common occurrence for cocaine, opiate, and PCP users with combinations of cocaine, opiate, and marijuana occurring most frequently.

Key Words: Drug Survey, Urine Testing, Immunoassay.

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Concentrations of Testosterone, ACTH, Cortisol, Prolactin, β-Endorphin, and Serotonin of Long Time Opiate Addicts

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In 1974, the concentration of testosterone of 102 male opiate addicts who were using methadone as a substitute was determined and compared to the concentration of 29 healthy male students of the same age. The subjects were between 20 and 31 years old (mean: 23.1). The results showed that in the morning the mean concentration of testosterone in the addicts was 438 ng/100 ml, and in the healthy subjects, 700 ng/100 ml. In 1993, the concentration of testosterone, ACTH, cortisol, serotonin, prolactin, β-endorphin, and serotonin of 40 subjects from the 1974 group of addicts were determined. Twenty-three were still opiate addicts; 17 were clean. The mean concentration of testosterone of the addicts was 400 ng/100 ml, and of the clean subjects, 650 ng/100 ml. The concentrations of ACTH, cortisol, prolactin, β-endorphin, and serotonin were not significantly different between the still addictive and the clean subjects.

Key Words: Hormones, Long Term Study, Opiate Addicts.

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Drugs and Alcohol Found in Fatal Civil Aviation Accidents Between 1989 and 1993

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Introduction: The Civil Aeromedical Institute (CAMI), Office of Aviation Medicine (OAM), Federal Aviation Administration (FAA) is tasked under public law 100-591[H.R. 4686]; November 3, 1988, AVIATION SAFETY RESEARCH ACT OF 1988, to conduct toxicological tests in aviation accidents and determine the effects of drugs on human performance. It is important for the FAA to identify the extent to which drugs and alcohol are being used by pilots involved in aviation accidents so that the FAA can take steps to prevent pilots from using drugs or alcohol, which would impair their ability to fly an aircraft. The toxicology reports prepared by the Forensic Toxicology Research Section are used by the FAA and the National Transportation Safety Board (NTSB) to determine the cause of aviation accidents and in civil and criminal litigation.

Method: Specimens (blood, urine, liver, kidney, vitreous and other bodily specimens) were collected by pathologists near the accident and placed in evidence containers provided by CAMI. Those samples were refrigerated and shipped by overnight air. Upon receipt the specimens were inventoried and accessioned for the analysis of drugs, alcohol, carbon monoxide and cyanide. All data collected by the laboratory were electronically entered into a computer for future analysis. The data base was searched using a program developed by the Forensic Toxicology Research Section. The data base was sorted based on the class of drug, controlled dangerous substance (CDS) schedules I and II, CDS schedules III-V, prescription drugs, over- the-counter drugs and alcohol.

Results: The Toxicology and Accident Research Laboratory received specimens from 1845 pilots for postmortem toxicology analysis between 1989 to 1993. CDS (schedules I and II) were found In 71 of the pilots analyzed. CDS (schedules III - V) were found in 27 of the pilots tested. Prescription drugs were found in 109 of the pilots analyzed. Over-the-counter drugs were found in 208 of the pilots analyzed. Alcohol at or above the legal limit of 0.04% was found in 147 pilots analyzed. The number of positive drug cases has doubled over the past 5 years.

Conclusion: Over-the-counter medications are the most frequently found drugs in fatal aviation accidents and many of these drugs, or the medical conditions for which they are being used, could impair a pilot's ability to fly an aircraft. The increased number of positive cases found in this research is most likely the result of improved methods of analysis rather than an increase in the use of drugs. The low incidence of CDS III-V drugs found in fatal aviation accidents may be a result of the difficulty in detecting and identifying the new benzodiazepines commonly prescribed in this class.

Key Words: Aviation, Drugs, Alcohol.

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The Analysis of Several Xanthines and Other Compounds in Human Plasma and Urine by Capillary High-Performance Liquid Chromatography/Mass Spectrometry

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Several compounds including xanthines (theophylline (TH), theobromine (TB) and caffeine (CA)) were determined in biological samples by gradient capillary high-performance liquid chromatography (HPLC)/fast atom bombardment mass spectrometry (FAB-MS) (LC/FAB-MS). 7-Ethyltheophylline was used as the internal standard (IS). An injected sample was trapped on a pre-concentration column. The valve was then switched to elute the effluent onto the separation column (flow rate: 4μL/min), allowing all the sample to be introduced to the MS without the sample being divided. Human deproteinized plasma and urine were absorbed on an Extrelut® column and recovered by eluting with dichloromethane. The positive FAB mass spectra of xanthines showed a peak at m/z M+H (which was the base peak for all), a peak at m/z M+H+Gly (matrix), and also some small fragment peaks. Xanthines were detected in almost all plasma and urine samples. Several other unidentified compounds were also detected. The relative retention time to IS was calculated. Some peaks (e.g., base peak: 188, 211, 415, etc.) were detected at the same relative retention time in almost all samples; this was likely due to an endogenous origin. Many drugs could be detected by this method. This procedure with LC/MS was an effective and useful screening test.

Key Words: Capillary LC/FAB-MS, Xanthines, Blood.

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Cocaine Contamination of United States Currency

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The exchange of illicit cocaine for money by drug dealers is an everyday occurrence in cities in the United States. There is ample opportunity during the exchange, storage and use of cocaine, for paper currency to become contaminated. Since currency is exchanged frequently, it is likely that contaminated currency would be found in common use. We examined 10 single dollar bills from several cities in the United States for the presence of cocaine. Individual bills were extracted with methanol (10 mL). Cocaine was purified from the methanol extract by solid phase extraction (SPE). The SPE extract was analyzed by gas chromatography/mass spectrometry. Standard curves were constructed with new, uncirculated currency. Cocaine was identified qualitatively by full scan and quantitated by selected ion monitoring. Results indicating that currency in general use is extensively contaminated are shown in the following table.

City	# Positive (>0.1 µg/bill)	# Positive (>1.0 µg/bill)	Max. Cocaine/Bill (µg/bill)
Baltimore, MD	9	9	522
Chicago, IL	7	3	2
Honolulu, HI	10	5	10
Kansas City, KS	9	8	24
Las Vegas, NV	9	5	14
Los Angeles, CA	9	6	11
Minneapolis, MN	8	5	434
Mobile, AL	9	7	70

Key Words: Cocaine, Currency, Environmental Contamination.

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Internally Concealed Cocaine: Analytical and Diagnostic Aspects

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Cocaine and benzoylecgonine were determined in the urine and saliva of 30 persons arrested at Frankfurt airport following the smuggling of internally concealed drug. The amount of cocaine hydrochloride found in an individual ranged from 400 to 1340 g, divided into containers weighing 4 to 13 g. The number of containers ranged from 43 to 135 per person. Fifteen urine samples showed positive immunoassay results (EMIT) and contained 0.04 - 6.75 mg/l BE (HPLC). All saliva samples were negative for cocaine. The leaching of cocaine from particular containers was investigated in 0.9% NaCl, in 0.1 HCl/pepsin and in trypsin solution (pH 8) by incubating for 48 hours in a stirring bath. The results of these experiments correlated with the results of urine examination.

Containers with cocaine, which were machine-made, showed large variability in regard to the number of layers (4 - 7), size, weight, and cocaine purity. This may be helpful in identifying the origin. The lowering of the cutoff-level of BE to 150 ng/mL in urine was proposed, in order to enhance the sensitivity of the screening EMIT procedure.

Key Words: Cocaine, Body Packing, Immunological Diagnostics.

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Studies on Canine Specificity and Sensitivity for Detection of Cocaine on Currency

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Previous studies have demonstrated the ubiquitous contamination of U.S. currency with cocaine. Since narcotics detecting dogs are often used to detect cocaine residue on currency, it was of interest to determine canine specificity and sensitivity for detection of cocaine-contaminated currency. Studies were conducted with canine teams from the Fayetteville, Arkansas Police Department utilizing uncirculated currency contaminated with 1-5000 µg of cocaine. Negative controls consisted of uncirculated drug-free currency as determined by GC/MS. In the initial study, all three dogs alerted to three of the four negative controls. Dog #1 also alerted to almost all of the cocaine currency ranging from 1-1000 µg. Dog #2 alerted to currency contaminated in the 200-1000 µg range. Dog #3 failed to alert to most of the contaminated bills except for the 500 and 1000 µg bill. Since the initial study showed that this group of dogs alerted to uncontaminated currency, a second experiment was conducted using plain bond paper contaminated with 5-5000 µg of cocaine. Clean bond paper served as a negative control. All four dogs did not alert to clean bond paper. Dogs #1 and 3 failed to alert to all levels of contaminated paper. Dog #2 alerted to paper contaminated with low and high but not intermediate levels of cocaine. Dog #4 only alerted to 200 and 5000 µg contaminated paper. A third experiment with the same group of dogs yielded similar results. These preliminary studies demonstrate that this group of dogs falsely alert to drug-free currency and therefore calls into question the significance of their alert to currency. The bond paper results show that considerable variability exists from dog to dog with respect to detection levels of cocaine. Moreover, no clear dose-response relationship was evident for these dogs under these conditions. Further studies are planned to determine if these results reflect a general phenomenon among narcotics detecting canine teams.

Key Words: Cocaine, Currency, Dogs.

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Comparison of Ethanol Concentrations in Blood, Serum and Blood Cells for Forensic Application

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Alcohol concentrations in serum (SAC) and whole blood (BAC) were determined for 235 subjects by a headspace gas chromatography method. The SAC:BAC ratio ranged from 1.04 to 1.26, with a mean of 1.14, and a normal distribution with a standard deviation of 0.041. Using a conversion factor for SAC to BAC of 1.18 (mean + I standard deviation), 84% of estimated BAC's were less than that measured; the remaining estimates differed by less than 7 mg/dL. A SAC >100 mg/dl indicated a BAC >80 mg/dL.

Alcohol concentrations in blood cells (CAC) were similarly determined for 167 subjects. The CAC:BAC ratio ranged from 0.66 to 1.00, with a mean of 0.865, and a positively-skewed normal distribution with a standard deviation of 0.065. Using a conversion factor for CAC to BAC of 0.93 (mean + 1 standard deviation), 89% of estimated BAC's were less than that measured; the remaining estimates differed by less than 8 mg/dL. A CAC >80 mg/dL indicated a BAC >80 mg/dL. The CAC is useful in forensic practice when either serum or whole blood are not available.

Key Words: Alcohol, Serum, Blood Cells.

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Comparison of Hospital Laboratory Serum Alcohol Obtained by an Enzymatic Method with Whole Blood Values Determined by Gas Chromatography

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The difference in hospital laboratory serum alcohol values compared to those determined in whole blood has been an issue addressed by articles in both the clinical and forensic literature. Clinical records of serum alcohol have been subpoenaed for court use in numerous DUI cases. The calculation of a factor to convert serum to whole blood has been achieved previously using serum and whole blood values both obtained by gas chromatography. A more realistic scenario in many states is the necessity of converting values obtained on serum by enzymatic methods to whole blood equivalents, since few medical facilities have a gas chromatograph devoted to alcohol determinations.

In this study, serum and whole blood samples were collected simultaneously and analyzed for ethanol using the DuPont ACA and a gas chromatographic procedure used in a forensic laboratory. More than 150 specimens were compared. The average serum/whole blood ratio obtained was similar to those values reported in the literature for gas chromatography studies alone. However, the importance of method bias elimination by stringent calibration requirements for both techniques is crucial to consistent results.

Key Words: Serum, Whole Blood, Ethanol.

mean 1.14 (range 1.06-1.24)

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Internal Versus External Retention Index Standards In Gas Chromatographic Drug Screening

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The gas chromatographic retention index methods reported in the literature nearly exclusively use the external standard method; the standard series is injected daily between a sequence of analysis samples. The internal standard method, on the other hand, involves the injection of the standard series with each sample. The former method allows the use of actual drugs as standards, while the latter method is able to compensate for chromatographic variations in each run. There is very little comparative information on the precision obtained by these methods with different sets of standards.

In the present study, the external and internal standard methods were evaluated in the screening for seven basic drugs on NB-54 and DB-1701 capillary columns with a homologous series of *N,N*-dialkylbenzylamines and with a series of basic drug substances. However, the drug series was not evaluated by the internal standard method as the standards are commercially available drugs and thus may be present in the sample. In the within-day study, the precision of the internal benzylamine series proved to be superior to both external standard series studied. In the day-to-day study, the internal benzylamine series on NB-54 was the best of the three, and on DB-1701 it was nearly as good as the external drug series. The external benzylamine series had the lowest precision in the day-to-day study. These findings suggest that further development of retention index methods seeking high precision should be based solely on the internal standard method.

Key Words: Gas Chromatography, Drug Screening, Retention Index.

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Identification of Urinary Clomipramine Metabolites By REMEDi HS in the Presence of Other Drugs

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Clomipramine is a tricyclic antidepressant which was recently approved in the U. S. for the treatment of obsessive/compulsive disorders. There have been many studies concentrating on the identification of clomipramine in serum and plasma samples by using HPLC with an off-line liquid-liquid extraction and a single UV wavelength detection. The compounds monitored in HPLC include the parent clomipramine [1,2], desmethylclomipramine [1,2], and 8-hydroxy metabolites [2]. A GC/MS method was reported for the detection of clomipramine and desmethylclomipramine in the extracts of whole blood [3]. Urine metabolites of clomipramine have not been extensively investigated.

In this study, we report that an automated drug screening HPLC system (REMEDi HS) can be used to identify clomipramine and its metabolites in urine samples. The REMEDi HS uses a multi-column on-line sample purification and separation technology with computer-aided identification of over 500 drugs and metabolites. A full UV spectrum (205 nm to 300 nm) is used for comparison in the drug identification software. In the patient urine samples, the REMEDi HS identified desmethylclomipramine, clomipramine-N-oxide and multiple 8-hydroxy metabolites in the presence of other drugs and metabolites, including thioridazine, mesoridazine, thioridazine-2-sulfoxide, diphenhydramine, and trimethoprim. GC/MS was used to confirm the presence of clomipramine and its metabolites; the parent compound is not always observed.

The capability to identify multiple drugs is useful for monitoring patient compliance and for avoiding the potential adverse effects due to drug interactions. For example, it has been reported that a concomitant administration of clomipramine with tranylcypromine (a monoamine oxidase inhibitor) is especially hazardous [4]. Care should be taken when administering clomipramine to patients taking antihypertensive medications like clonidine [5]. The REMEDi HS can identify clomipramine and its metabolites in the presence of tranylcypromine or clonidine because of their different retention indices and characteristic UV spectra.

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Key Words: Urinary Metabolites, Clomipramine, REMEDi HS.

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Simultaneous Analysis of Opiates, Amphetamines, Benzodiazepines, Cocaine and Metabolites in Urine by GC/MS Using Pentafluoropropionic Anhydride and Pentafluoropropanol Derivatization

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We describe a GC/MS method for the identification of opiates, some amphetamines, some benzodiazepines, cocaine and cocaine metabolites in urine after ChemElut extraction and pentafluoropropionic anhydride and pentafluoropropanol derivatization.

Hydrolysis was performed by adding 40 µL of the crude solution of Helix Pomatia in 2 mL of urine. The pH was adjusted to 4.5-5 and the sample was hydrolyzed in 37° C for 24 h. After hydrolysis the pH was adjusted to 8-9 and the drugs were extracted from urine using ChemElut extraction columns with 2 x 6 mL of dichloromethane:2-propanol (9:1).

After extraction the organic solvent was evaporated to dryness. Pentafluoropropionic anhydride (50 µL) and pentafluoropropanol (25 µl) were added and the tubes were mixed and tightly capped. The tubes were heated in the waterbath (60°-65°) for 30 min. Excess reagent was evaporated and the residue was dissolved in toluene (80 µl).

Derivatization of benzoylecgonine with PFPA (reacts with phenolic hydroxy and amino groups) and PFPOH (reacts with acidic groups) was necessary to convert the polar functional groups into nonpolar derivatives and to make the analyte suitable for gas chromatography.

An HP 5890 gas chromatograph connected to HP 5970 mass selective detector was used for analysis. The chromatographic column was 12 m HP-1, 0.2 mm i.d., with 0.33 µm film thickness. Injection (1 µL) was done in the splitless mode. The oven temperature was held at 60° C for 1 min and then increased up to 300° C at the rate of 30° C/min (held for 2 min).

The described method is suitable for confirmative analysis of opiates, some amphetamines, some benzodiazepines, cocaine and cocaine metabolites.

Key Words: GC/MS, Pentafluoropropionic Anhydride and Pentafluoropropanol Derivatization.

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Improved Methods for Detecting Felbamate by Thin-Layer Chromatography

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Last year we had a case involving the death of a 24-year-old single white male where the autopsy findings revealed the cause of death was due to an idiopathic seizure disorder. The deceased was reported to have been treated with Felbamate (Felbatol™), a new dicarbamate antiepileptic drug which was introduced in 1993. Postmortem toxicology revealed the blood analysis for felbamate by GC/MS was positive (13.3 µg/mL). Peak steady-state plasma concentrations are proportional to the dose and begin at 17 µg/mL for a dose of 15 mg/kg/day.

Toxi-Lab A™ Thin-Layer Chromatography Drug Detection System was employed as a urine screening method. However, we found the detection of felbamate using the Toxi-Lab A system alone was difficult. Felbamate is 22-25% human plasma protein bound. Over 90% of the absorbed dose is recovered in the urine. Approximately 40-50% occurs as unchanged drug and 40% as unidentified metabolites and conjugates. In an attempt to improve the detection of felbamate, several simple preparation techniques prior to using Toxi-Lab A were examined. The techniques examined included the use of acid hydrolysis, enzymatic hydrolysis, acetone and methanol protein free filtrate, and TDx precipitate reagent.

The acetone and methanol preparation methods gave the best recovery of felbamate when detecting with the Toxi-Lab A system. These methods involved adding 2 mL of acetone or methanol to 3 mL of the urine sample, vortexing and adjusting the pH to match the urine sample. The tubes were centrifuged at 3400 rpm's for 5 minutes and the filtrate was collected to run Toxi-Lab A. Employing the more desirable acetone preparation method, results for detecting felbamate using the Toxi-Lab carbamate confirmation technique were obtained.

Key Words: Felbamate, Thin-Layer Chromatography, Toxi-Lab™.

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New Approaches to the Analyses of Biological Matrices Using Inductively Coupled Plasma Spectrometry

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Comprehensive tissue analysis provides critical information to researchers and forensic scientists. Until recently the interest of the clinical community was focused on single element analyses. Today, however, growing attention is paid to panels of 5-10 elements. Graphite furnace atomic absorption spectrometry (GFAAS) has routinely been used to detect trace elements in body fluids. However, GFAAS requires a high degree of operator skill, the useful analytical range is very limited, it is prone to spectral interferences, it is a single element technique with poor sample throughput, and matrix modifiers are required. Biological samples typically contain widely divergent levels of analytes, high levels of dissolved salts, and organic compounds, which can cause matrix effects or spectral interferences in GFAAS. This presentation will demonstrate the applicability of the multi-element techniques of Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with an axially-viewed plasma and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), to the analysis of urine. Sample preparation techniques, analytical results for standard reference materials (SRM), spiked recoveries, detection limits, and long term stability will be discussed for each technique.

For ICP-OES the detection limits in urine ranged from 0.4 ppb to 89 ppb for the elements of interest Three SRM's were analyzed and compared with certified values within 85% or better for all elements reported. Spiked recoveries for SRM urine specimens and typical urine samples fortified at levels from 0.5 to 10 ppm ranged from ≥ 80%. For ICP-MS, the detection limits ranged from 0.1 ppb in diluted urine. SRM results varied from 84% of theoretical or better. Likewise, spiked recoveries for samples fortified at levels from 1 to 10 ppb ranged from 80%.

Key Words: Biological Fluids, ICP-MS, ICP-OES.

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Ion Mobility Spectrometry (IMS) Detection of Cocaine in Hair

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The rapid analysis of hair samples contaminated with cocaine is explored with IMS detection. Subsequent data analysis of analyte desorption profiles can differentiate between internal and external contamination. IMS is both specific and sensitive to most narcotics. Typical IONSCAN cocaine sensitivity is subnanogram.

The use of a Barringer IONSCAN IMS, which is comprised of a thermal desorption sample introduction system and IMS permits direct hair analyses to be made in less than five seconds for up to 18 analytes simultaneously. Thermal desorption and high sensitivity allows data to be obtained from single strands of hair only 10 mm in length.

Differentiating internal and external contamination is made possible, when desorbing from a wire mesh filter, the analyte intensity vs. time desorption profiles show marked differences. Further investigations are now underway with additional hair samples contaminated with other narcotics. Development of methodology for quantification is also being pursued.

IONSCAN analyses of hair, when performed in real-time, may prove very valuable in addressing the arguments of both environmental contamination and chain of custody issues.

Key Words: IMS, Cocaine, Hair.

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Rapid Screening Test for the Detection of Drugs of Abuse in Urine

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We present a new semiquantitative immunoassay screening test for the detection of drugs of abuse in urine. The immunoassay is performed with dried reagents on a chromatographic test strip using the Gloria (gold labelled optical read immuno assay) technology. It is the first one step screening assay for drugs of abuse. The test strip is dipped into the urine for 5 sec and then left for colour development. The result can be read after 2 min by comparing the red reaction colour developed in the detection area with a comparison scale on the vial.

The test strip results are in good agreement with other commonly used automated immunoassay methods. With GC-MS as a reference method, we found the following data for sensitivity and specificity in four evaluation sites:

	Sensitivity	Specificity
Cocaine	>97%	>98%
Cannabis	>90%	>93%
Opiate	>96%	100%

With regard to crossreactivity, we found positive results only with BZE and free cocaine for Frontline® cocaine, with 11-Nor-Δ9 (and Δ8)-THC-carboxy acid in low, and the other THC metabolites in high concentrations for Frontline® cannabis, while nearly all opiate metabolites gave positive results with Frontline® opiate.

Using the new Frontline® test strips the determination of cocaine, cannabis, opiates, and their respective metabolites is easy to perform without any pretreatment of the urine or reagent handling and gives results within 2 min.

Key Words: Gloria, Drugs of Abuse Testing in Urine.

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Radiative Energy Attenuation Ethanol Testing on the Abbott Random/Continuous Access Immunoassay Analyzer

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Radiative energy attenuation (REA®) is a technology that measures color development using a fluorescence detection optical system (e.g., FPIA detector of Abbott TDx®, ADx®, and TDxFLx® analyzers). A fluorophore (e.g., fluorescein) is introduced which is stable under test conditions. The reaction system uses analyte to cause a proportional change in concentration of a chromophore. Measured fluorescent energy of the fluorophore is modulated by the chromophore through absorption of activation and/or emission energy. A homogeneous coupled enzyme reaction system is currently used to enable REA detection of ethanol in biological fluids on the Abbott TDx, ADx and TDxFLx analyzers.

In this study, we have conducted prototype evaluation of an REA Ethanol protocol on the Abbott Random/Continuous Access Analyzer (AxSYM®). Reagents from the existing TDx/ADx assays were used without modification in the AxSYM analyzer to assess calibration curve suitability, sample matrix effects, sample/analyte stability and reagent stability. Throughput estimates were obtained with acceptable assay protocols that allowed for >80 tests per hour in both batch and mixed assay load lists. Precision of less than 6% within run and sensitivity of less than 10 mg/dL were achieved. Time to first result was less than 20 minutes. Calibration curve ranges were the same as that used on TDx and ADx: 0 to 300 mg/dL. Ethanol recovery from both serum controls and whole blood controls was within recommended ranges for use on TDx and ADx. Sample correlation to TDx was: slope = 0.93, intercept =12.2, correlation coefficient = 0.971 (N=23).

We conclude that this analyzer is capable of acceptable performance for measurement of ethanol by REA, while operating in continuous/random access or batch operation mode.

Key Words: Ethanol, AxSYM, Radiative Energy Attenuation.

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More Fatal Zipeprol and/or Dextromethorphan Poisonings in Korea

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The abuse of zipeprol, an antitussive agent, has continued among young people in Korea. To obtain a stronger hallucinatory effect, there is a growing tendency to abuse this drug with dextromethorphan, which is also an antitussive agent. Because individuals self-administer both drugs in high doses, fatalities also have increased. Since 1993, 24 zipeprol and/or dextromethorphan fatal poisonings were identified due to the analysis of postmortem blood and gastric contents of victims who had a history of drug abuse. (The authors already had reported 23 fatal cases involving zipeprol in 1992.)

GC/TSD and GC/MS were used for the determination and identification of drugs. Linear calibration curves and high recoveries were obtained by this method.

Among 24 cases, zipeprol was identified in 15 cases, while both zipeprol and dextromethorphan were detected in 9 cases. The blood zipeprol concentrations varied from 1.3 to 28.6 μg/mL; whereas, dextromethorphan concentrations ranged from 1.1 to 18.3 μg/mL. The ratios of gastric contents versus blood concentration of zipeprol ranged from 1.9 to 914.0 in 20 cases and ratios of dextromethorphan were 1.2 to 203.1 in 4 cases.

Key Words: Zipeprol, Dextromethorphan, Blood and Gastric Contents.

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Abuscreen OnLine® Immunoassays for the Detection of Drugs of Abuse on the Cobas INTEGRA® Analyzer

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Applications for the Abuscreen OnLine DAT tests (amphetamine, barbiturate, benzodiazepine, cocaine, methadone, opiate, PCP, and THC) were developed on a new, fully automated clinical chemistry analyzer, the Cobas Integra. This random access analyzer has the capacity to consolidate the testing of clinical chemistries, therapeutic drugs, drugs of abuse, specific proteins, thyroids, and electrolytes on one system. It has a throughput capability of 600 tests per hour, up to 860 tests per hour with ISE. The OnLine assay system is based on the Kinetic Interaction of Microparticles in a Solution (KIMS) where the drug concentration in a urine sample is directly proportional to the inhibition of microparticle aggregation.

Abuscreen OnLine reagents were adapted to the Cobas Integra's novel reagent cassette (200 tests per cassette) resulting in automatic reagent handling and requiring no manual reagent preparation, whatsoever. This unique reagent packaging allows up to one (1) month on-board (open vial) calibration curve stability and at least two (2) months on-board reagent stability with recalibration. Results are obtained in ng/mL from a standard curve that is calculated using four calibrator levels. Samples containing analyte at concentrations above the cut-off are flagged as "positive".

Using the Online technology on the Cobas Integra, the following precision results were obtained. Quantitative intra (n=25) and inter assay precision, X=NIDA cut-off: typical CV's of ≤ 5.0% at the 0.8X, 1.0X and 1.2X. The above OnLine immunoassays have a greater than 99% confidence level that the 0.8X is negative and the 1.2X is positive. Patient correlation studies (150 samples per assay) against the reference method of GC/MS and the Syva Emit technology demonstrated clinical efficacy on the Cobas Integra for all analytes.

The Cobas Integra's state of the art UNIX based windows software with user friendly graphics provides convenient operator use and data management and a flexibility to adapt to the diverse array of clinical laboratories and their specific needs.

Key Words: Integra, Microparticles, Drugs of Abuse.

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A Case of Manganese Poisoning During Long-Term Total Parenteral Nutrition

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A case of manganese (Mn) poisoning occurred in a female under total parenteral nutrition (TPN) for several months. Autopsy samples (including brain, myocardium, kidney, liver, and squelettic muscle) were digested with $HNO_3/HClO_4$ (50:50, v/v), then assayed for Mn by GFAAS with Zeeman correction. Postmortem tissue samples from 3 control subjects who died under other circumstances were analyzed in the same manner. Data (see Table) show significant accumulation of Mn in the brain samples of the patient under TPN. Our results are discussed in the light of the existing literature.

Manganese Levels In Tissue Samples (all values in µg/g wet tissue)

	Control 1	Control 2	Control 3	TPN Patient
Globus pallidus	0.43	0.40	0.33	0.95
Putamen	0.55	0.43	0.32	1.00
Nucleus caudatus	0.56	0.42	0.41	1.22
Centrum ovale	0.37	0.31	0.27	0.71
5th gyrus temporalis	0.32	0.22	0.27	0.44
3rd gyrus frontalis	0.27	0.18	0.21	0.39
Thalamus	0.38	0.28	--	--
Cerebellum	--	--	--	0.43
Myocardium	--	--	--	0.40
Kidney	--	--	--	1.30
Liver	--	--	--	4.75
Squel. muscle (psoas)	--	--	--	0.20

Key Words: Manganese, Poisoning, Total Parenteral Nutrition.

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Assay of Amphetamines by Roche Online® Immunoassay with Periodate Oxidation on the Hitachi 717

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Even with the increased specificity of the Online® assay for amphetamine, high concentrations of phenylpropanolamine and to a lesser extent ephedrine, can result in a positive screening result. Sodium periodate is routinely employed to oxidize phenylpropanolamine and ephedrine at the alpha-hydroxy position to prevent interference with the GC/MS analysis of amphetamine and/or methamphetamine.

A 0.4 M solution of sodium periodate was prepared fresh daily and added to the Online microparticle reagent (B). The most effective concentration was 20 µL of sodium periodate solution to 1 mL of microparticle reagent. All calibrators, controls and samples were analyzed with this solution. The antibody diluent with buffer reagent (A) was not changed.

The periodate solution eliminated interference from phenylpropanolamine and ephedrine up to 1 mg/ml in the initial immunoassay for amphetamines. Good correlation of GC/MS and immunoassay results for amphetamine were achieved. The GC/MS instrument use time for amphetamine/methamphetamine has been reduced by 60% due to the elimination of false positives for amphetamines in the screening procedure.

Key Words: Amphetamine, Sodium Periodate, Immunoassay.

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Detoxification of Xenobiotics Containing the Carboxylic Acid Group: Relationship Between Structure And Glycine Conjugation

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Xenobiotics having the carboxylic acid group are very commonly encountered and are of significance as drugs, herbicides and insecticides. In addition, many compounds are readily converted to carboxylic acids by metabolism. For example, toluene is oxidized to benzoic acid. Glycine conjugation is the most important route in the metabolism of carboxylic acids. Correlations between molecular structure and glycine conjugation are also important in order to understand detoxification mechanisms. Therefore, in this study glycine conjugation of a series of substituted benzoic acids was investigated in rat, mouse and bovine liver and kidney mitochondria. Various acids and their glycine conjugates were analyzed by HPLC. Using this method, we defined HPLC chromatographic properties of substituted benzoic acids and their glycine conjugates. Calculation of the relative retention time (t_R) value will permit provisional identification of an unknown glycine conjugate. In addition. the extent of glycine conjugation of substituted benzoic acid in liver mitochondria was different from that in kidney mitochondria. Glycine conjugation of the meta-isomers particularly varied with species. The lipophilicity of the chemicals and the size of the substituents had a great influence over glycine conjugation. These findings are useful for the detection and identification of glycine conjugates in the field of forensic toxicology.

Key Words: Glycine Conjugation, Structure-Metabolism Relationship, HPLC Chromatographic Property.

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Comparison of AxSYM's TDM Carbamazepine, Phenobarbital, Phenytoin and Valproic Acid Assays with Abbott's TDx Assays

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Abbott's AxSYM System is a large, automated random/continuous access immunoassay analyzer. We report the comparison of Abbott's AxSYM System and the TDx Analyzer in the measurement of carbamazepine, phenobarbital, phenytoin and valproic acid. AxSYM and TDx use the same fluorescent polarization immunoassay technology. Many patients with seizure disorders receive one or more of these drugs. The AxSYM System can measure one or more analytes from a patient sample or samples in a random testing manner.

Precision studies on Abbott's AxSYM for the four antiepileptic drugs were performed with tri-level quality control material over 20 days and demonstrated the following results:

	Coefficient of Variation		
Analyte	Subtherapeutic	Therapeutic	Elevated
Carbamazepine	3.79%	3.12%	3.56%
Phenobarbital	2.04%	2.02%	2.98%
Phenytoin	3.53%	2.64%	2.66%
Valproic Acid	3.45%	3.29%	3.46%

Patient specimen correlation studies between the AxSYM System and the TDx analyzer for the four antiepileptic drugs gave the following data:

Analyte	N	Correlation Coefficient	Slope	Y-Intercept
Carbamazepine	100	0.967	0.982	0.35
Phenobarbital	100	0.995	0.978	-0.37
Phenytoin	102	0.997	0.997	-0.08
Valproic Acid	102	0.992	0.992	-0.14

The AxSYM System demonstrated reliable precision data and was comparable to the Abbott TDx in patient specimen testing for carbamazepine, phenobarbital, phenytoin and valproic acid.

Key Words: AxSYM, Immunoassay Analyzer, Antiepileptic Drugs.

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MDMA and Related Compounds in Human Urine from a Controlled Study: Comparison and Validation of Different Immunoassays

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The illegal consumption of 3,4-methylenedioxymethamphetamine (MDMA), a psychoactive phenylalkylamine called "Ecstasy", has gained popularity not only in Switzerland but also in other European countries. In Switzerland, MDMA is increasingly used medically as an adjunct in psychotherapy.

It was the aim of the present study to examine the efficiency of different amphetamine/methamphetamine immunoassays (Abuscreen ONLINE, TDx, etc.) for the detection of MDMA and its metabolites in human urine. In a controlled study, six patients received 1.5 mg/kg MDMA orally. The urine samples were collected over a period of 72 hours. The screening was performed with different commercially available immunoassays. The immunoassay data were compared with those of HPLC-DAD and GC/MS.

The cross-reactivity for MDMA is concentration dependent and ranges from 0.3 - 50% for Abuscreen ONLINE and 18 -118% for TDx, respectively. For one of the immunoassays (Abuscreen ONLINE) further experiments concerning the cross-reactivity of MDMA and its metabolites 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA) have been performed. In the presence of MDMA and MDA, a synergistic effect and a concentration dependent cross-reactivity from 21% to 100% were found.

Key Words: MDMA, Immunoassay, HPLC-DAD / GC-MS.

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Performance Comparison of Syva EMIT® II and Roche Abuscreen Online® with Abbott TDx Assays for Drug Screening

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The performance of Syva EMIT® II and Roche Online® were evaluated for amphetamine, barbiturate, benzodiazepine, cannabinoids, methadone, opiate and PCP. All tests were run on the Hitachi 717 and included 86 positive patient urine samples. In addition, forty CAP proficiency samples from 1992-UDS and 1993-UDS surveys were also analyzed. All screen positives were analyzed by TDx assay, except methadone and PCP. All positive results were confirmed by GC/MS.

The positive detection rate for EMIT II and Online was equivalent for cocaine, opiate and PCP. Variable detection rates were seen for the other analytes.

For barbiturates, Online and TDx detected 19 patient positives while EMIT II detected 18 (1 false negative). All three assays detected 3 CAP positives. For benzodiazepines, EMIT detected 21 patient positives while Online and TDx detected 18 (3 false negatives). All three assays detected 2 CAP positives. For methadone, EMIT detected 1 positive patient while Online detected 4 (three false positives).

For amphetamine, EMIT and Online detected 2 true patient positives with 3 EMIT false positives and 2 Online false positives. TDx detected only the 2 true positives. For CAP samples with only amphetamine and/or methamphetamine present, EMIT detected 5 true positives, Online detected 4 true positives with 1 false negative and 2 false positives. TDx detected 5 true positives and 3 false positives.

For cannabinoids, with a 50 ng/ml cut-off, EMIT detected 91% (40/44), Online 75% (33/44) and TDx 100% (44/44). With a 100 ng/ml cut-off, EMIT detected 100%, Online 78% (18/23) and TDx 91% (21/23). All assays detected 5 CAP positives.

In conclusion, while the three assays are comparable, differences in the positive detection rate exists between the three.

Key Words: Drug Screening, Positive Detection Rate, Performance Comparison.

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Screening for Acidic and Neutral Drugs by Instrumental Qualitative Thin-Layer Chromatography

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Recently, a new concept for qualitative analysis by instrumental TLC was reported: correction of R_f values by the polygonal method with several standards, and searching libraries using the corrected R_f values (hR_fc) and UV spectra. The method has proved to be powerful in the screening for basic drugs in autopsy samples.

The present paper describes a screening procedure for acidic and neutral drugs in autopsy liver samples. After a solvent extraction, the drugs are analysed by RP-18 reversed phase TLC with the mobile phase methanol:water, 65:35. This system produces precise chromatography even with drugs that frequently cause problems in normal phase TLC, such as carboxylic acids, benzodiazepines and xanthine derivatives. Identification is based on instrumental qualitative TLC, with use of four correction standards and a library consisting of one hundred acidic and neutral drugs.

Key Words: Drug Screening, Instrumental TLC, Reversed Phase TLC.

106

Screening of Meconium Samples for Abused Drugs with a Modified Emit II Reagent

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The purpose of this project was to evaluate the analytical sensitivity of the Emit II assay system for screening meconium samples for abused drugs. One gram samples of meconium were extracted with 5 mL of 0.1 mol/L phosphate buffer, pH 6:methanol, 4:1. After low speed centrifugation the extracts were assayed in the same manner as for urine with the following exception: Reagent 1, which contains the antibody, was diluted with two parts of a Reagent 1 Diluent that was supplied by Syva. Decreasing amounts of methamphetamine, morphine glucuronide, benzoylecgonine, oxazepam, THCCOOH and secobarbital were added to an extract of pooled, drug-free meconium. The extracts were assayed and a response curve was generated for each drug. Twenty different drug-free meconium samples were also extracted, assayed, and the mean and SD of the rate for each drug assay was calculated. A minimum detectable dose was determined by finding the drug concentration on the response curve that corresponded to the mean + 3 SD rate found for the drug-free samples.

	Minimum Detectable Dose	
Drug	ng/mL Extract	ng/g Meconium
Methamphetamine	200	1200
Benzoylecgonine	60	360
Morphine Glucuronide	30	180
Secobarbital	60	360
Oxazepam	40	240
THCCOOH	50	300

Key Words: Meconium, Abused Drugs, Immunoassay.

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The Quantitation of Hydromorphone in Postmortem Specimens: Derivatization Prior To Extraction

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A new quantitative procedure has been developed for the analysis of hydromorphone in postmortem specimens. It has been used in 4 Medical Examiner's cases as described below. Hydromorphone, (Dilaudid™) is a semi-synthetic congener of morphine. Clinically, it is important for its use as an antitussive and an analgesic, however, it is particularly popular as a drug of abuse.

The analysis of hydromorphone in biological sample is complicated by poor extraction efficiency, low therapeutic levels and poor chromatographic behavior. These complications can be largely overcome by derivatization prior to extraction. Hydromorphone and the internal standard, d_3-hydromorphone, are derivatized in aqueous media with pentafluorobenzoyl chloride. The derivatized fluoro compounds are extracted in toluene and analyzed by gas chromatography/mass spectrometry using selected ion monitoring of the ions m/z 195, 423, 479 (hydromorphone) and 195, 426 and 482 (d_3-hydromorphone); quantitating on the 479 and 482 ions. The following table provides the tissue distribution of hydromorphone in four medical examiner cases:

	Blood (mg/L)	Urine (mg/L)	Bile (mg/L)
Case #1	0.14	2.28	n/a
Case #2	0.014	n/a	2.65
Case #3	0.05	n/a	0.27
Case #4	0.071	0.4	1.9

This method is sensitive, fast and specific for the detection and quantitation of hydromorphone in postmortem specimens.

Key Words: Hydromorphone, Pre-extraction Derivatization, GC/MS Analysis.

108

Qualitative Urine Screening for Tricyclic Antidepressants: Comparison of TRIAGE® Plus TCA Rapid Immunoassay Device with Gas Chromatography with Nitrogen-Phosphorus Detection

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The performance of the TRIAGE® Plus TCA Rapid Immunoassay Device was compared with that obtained using our current GC/NPD screening method to evaluate the advantages of using a rapid screening device for the detection of tricylic antidepressants in the urine.

TRIAGE® Plus TCA device is a rapid, qualitative monoclonal antibody-based competitive immunoassay that discretely screens for tricylic antidepressants in addition to a panel of 7 drugs of abuse. Since the antibody cross-reacts with a broad spectrum of tricyclic antidepressants, the drug detected is not identified by the device. We compared results from the tricyclic antidepressant channel of the TRIAGE® Plus TCA device with those obtained using our current GC/NPD screening method. Fifty-four urine samples obtained from hospital patients were initially screened for the presence of tricyclic antidepressants by GC/NPD. Comparison results are summarized below:

Positive by both methods:	26
Negative by both methods:	22
GC/NPD positive/TRIAGE+TCA® negative:	6

The TRIAGE® Plus TCA cutoff level for tricyclic antidepressants is 1000 ng/mL. The sensitivity limit of our GC/NPD method is 50 ng/mL for all of the tricyclic antidepressant drugs detected in this study. Six of the thirty-two positive samples tested contained tricyclic antidepressants at levels below the TRIAGE® Plus TCA cutoff level.

The TRIAGE® Plus TCA device is appropriate for the rapid, off site screening of concentrations of tricyclic antidepressants greater than 1000 ng/ml in urine, but will detect fewer urines positive for tricyclic antidepressants than our GC/NPD method using a 50 ng/ml sensitivity limit.

Key Words: Immunoassay, Triage, GC/NPD.

109

Effect of Common Adulterants on Roche OnLine® Reagents for Drugs of Abuse

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We tested the effect of table salt, Visine eye drops, bleach, Drano, and liquid soap on the OnLine reagents using the Cobas Mira Plus. In this system, an antibody-microparticle-drug complex forms aggregates when no drug is present. A substance that inhibits this aggregation will cause a positive test result.

We performed three experiments in which the adulterants were added to negative urine (experiment 1), urine with drug at 25% above cutoff (experiment 2), and positive urine from patients (experiment 3). In experiment 1, Drano caused a positive for amphetamines, PCP and opiates; bleach caused a positive for PCP; and liquid soap caused a positive for THC, benzodiazepines, amphetamines and PCP. In experiment 2, Drano caused THC to be negative. Liquid soap caused negative benzodiazepines, cocaine metabolite, opiates and barbiturates results. All other adulterants gave a positive result. In experiment 3, only Drano, Visine and table salt were used. A positive THC was changed to a negative by all three substances. A positive benzodiazepines was changed to a negative by Drano. All other positives remained positive.

In conclusion, some adulterants caused a false positive result, most adulterants
tested did not cause a false negative result, and Drano had the greatest effect on the test results.

Key Words: Drugs of Abuse Testing, Adulterants, KIMS.

110

Analysis of Drugs of Abuse in Human Whole Blood by Micro-Plate Enzyme Immunoassay (EIA)

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Untreated forensic whole blood samples were analysed for a range of commonly abused drugs using the STC Diagnostics Micro-Plate EIA system. The drug assays evaluated include amphetamines, cannabinoids, cocaine metabolites and opiates. The results obtained by this technique were compared with those obtained by RIA and other immunoassay techniques.

Key Words: Forensic Whole Blood Samples, Drugs of Abuse, Immunoassay.

111

Abuscreen OnLine® 100 Test Kit Immunoassays for the Detection of Methaqualone and Propoxyphene in Urine on the Cobas Mira® Analyzer

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Using the OnLine technology, a rapid, automated homogeneous immunoassay for the detection of methaqualone, propoxyphene and nor-propoxyphene in urine has been developed on the Cobas Mira analyzer. There is no need for sample pre-treatment and reagents are packaged in Mira compatible containers which may be used directly from storage at 2-8° C. These immunoassays are based on the Kinetic Interaction of Microparticles in Solution (KIMS) where the drug content in a urine sample is directly proportional to the inhibition of microparticle aggregation. The results for a sample are compared to a standard curve generated from four calibrators: 0, 150, 300 and 600 ng/mL methaqualone. Any sample determined to have equal to or greater than the cut-off of 300 ng/mL is flagged as > Normal Range.

The OnLine 100 test kit assay was evaluated for precision, sensitivity, selectivity, cross-reactivity, and urine interferences. Quantitative precision within-run (n=20, X=cut-off of 300 ng/mL), at 0.5X, 0.8X, 1.0X and 1.2X was ≤ 7% for both assays. Methaqualone positive patient samples could not be obtained; therefore, 50 negative urine samples were spiked with methaqualone at concentrations of 240 and 360 ng/mL. The assay correctly distinguished all positive from negative samples, relative to a 300 ng/mL cutoff. Cross-reactivity to unrelated compounds was ≤ 0.15%. Cross-reactivity to the primary metabolite in both assays was ≥ 80%. Interference studies showed no negligible effects due to creatinine (50 mg/dL), urea (200 mg/dL), protein (500 mg/dL) and hemoglobin (750 mg/dL).

The calibration curves for the OnLine Assay are stable for up to 30 days and the assay has a throughput of approximately 60 samples per hour.

Key Word: OnLine, Methaqualone, Propoxyphene.

112

Evaluation of the BIOSITE TRIAGE™ Plus TCA versus TOXI-LAB® for Detection of Tricyclic Antidepressants in Urine

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The BIOSITE Diagnostics TRIAGE™ is a point of care urine drugs of abuse testing device that simultaneously detects seven classes of drugs. TRIAGE™ provides a rapid and useful alternative to conventional immunoassay methods in both work place and emergency room urine drugs of abuse testing. However, like the currently available FDA approved urine immunoassay kits, the TRIAGE™ does not have the capability to detect tricyclic antidepressants (TCA). Recently, BIOSITE has developed the TRIAGE™ Plus TCA (TRIAGE™ Plus) which includes TCA. We evaluated the performance of the TCA component of TRIAGE™ Plus versus TOXI-LAB® thin layer chromatography system to detect positive urines containing TCA. Discrepant urines were confirmed for TCA by HPLC. Correlation between methods was 94% (n=49) with the three (3) discrepant urines being positive by TOXI-LAB for imipramine and/or desipramine and negative by TRIAGE™ Plus. HPLC confirmation determined imipramine and desipramine at 95 ng/ml and 347 ng/ml, respectively; desipramine at 287 ng/ml and the third urine was negative by HPLC for TCA. The imipramine/desipramine concentrations are below the 1000 ng/ml cutoff concentrations of TRIAGE™ Plus for these analytes. We conclude that the BIOSITE TRIAGE™ Plus would be useful for the rapid drug class identification of TCA in urine.

Key Words: TRIAGE™, Tricyclic Antidepressants, TOXI-LAB®.

113

Comparison of AxSYM's TDM Gentamicin, Theophylline and Tobramycin Assays with Abbott's TDx Assays

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Abbott's AxSYM System is a large, automated random/continuous access immunoassay analyzer. We report the comparison of Abbott's AxSYM System and the TDx Analyzer in the measurement of gentamicin, theophylline and tobramycin. AxSYM and TDx use the same fluorescent polarization immunoassay technology. The AxSYM can hold up to 20 different reagent packs. The AXSYM System can measure one or more analytes from a patient sample or samples in a random testing manner.

Precision studies on Abbott's AxSYM for the three therapeutic drugs were performed with tri-level quality control material over 20 days and demonstrated the following results:

Analyte	Coefficient of Variation		
	Subtherapeutic	Therapeutic	Elevated
Gentamicin	6.67%	2.79%	4.23%
Theophylline	3.13%	2.80%	3.98%
Tobramycin	3.98%	3.38%	4.26%

Patient specimen correlation studies between the AxSYM System and the TDx analyzer for the three therapeutic drugs gave the following data:

Analyte	N	Correlation Coefficient	Slope	Y-Intercept
Gentamicin	110	0.992	1.008	-0.02
Theophylline	103	0.990	1.006	-0.28
Tobramycin	109	0.995	1.024	-0.14

The AxSYM System demonstrated reliable precision data and was comparable to the Abbott TDx in patient specimen testing for gentamicin, theophylline, and tobramycin.

Key Words: AxSYM, Immunoassay Analyzer, Therapeutic Drugs.

114

Formation of Neurotoxic Metabolite Derived from Haloperidol in Brain and Liver Tissues

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Haloperidol (HP) is a clinically useful antipsychotic agent which has severe extrapyramidal side effects including Parkinsonism and, following chronic exposure, tardive dyskinesias. As it is structurally similar to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), it has been suggested that its side effects result from a similar mechanism. Recently, the detection of the neurotoxic pyridinium metabolite (HPP+), comparable to MPP+, derived from HP in the brain tissue of HP-treated rats has been reported. In order to investigate the formation pathway of HPP+ derived from HP in the brain tissue, we compared the activity of HPP+ formation in brain and liver. The HPP+ formation was determined as follows: 1) rat brain mitochondrial fractions or hepatic microsomal fractions were incubated with 20 pM HP and 0.1 M phosphate buffer, pH 7.4 in absence and presence of 1 mM NADPH at 37°C for 20 min. 2) HPP+ formed in the incubation medium was quantified by HPLC method with fluorescence detection. The activity of conversion of HP into HPP+, in brain was only a little, but in liver was about 300 times higher than in brain. The HPP+ formation from HP in liver was NADPH-dependent and catalyzed by cytochrome P-450. Moreover, the main cytochrome P-450 isoform involved in this pathway was determined to be CYP3A as a result of an immunoinhibition study. In conclusion, in contrast to the case of MPTP, the neurotoxic metabolite HPP+ detected in brain tissue of HP-treated rats was generated in liver and distributed to the brain.

Key Words: Neurotoxicity of Haloperidol, Haloperidol Metabolism, Pyridinium Metabolite of Haloperidol.

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Evaluation of the Triage® Panel for Drugs of Abuse Plus Tricyclic Antidepressants Test in Urine

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The Triage® Panel for Drugs of Abuse Test Plus Tricyclic Antidepressants is a 10 minute, solid phase, competitive immunoassay to simultaneously test a urine specimen for the presence of 8 drugs or drug metabolites in urine. The Triage device uses a drug-labeled colloidal gold conjugate to detect the presence of drugs in urine. An aliquot of urine is allowed to react with the lyophilized drug conjugate bead and a lyophilized monoclonal antibody bead for 10 minutes. If the drug of interest is present at or above the threshold concentration, the monoclonal antibody binds the drug and the labeled drug conjugate is available to bind to an antibody immobilized on a membrane. A positive result is indicated by the presence of a colored bar on the membrane adjacent to the name of the drug. In the absence of drug or if the drug is below the threshold concentration, no signal is observed on the device. The device incorporates a positive and negative procedural control to monitor sample and reagent integrity. The Triage device was compared to other analytical techniques for the detection of TCA's in urine. These techniques included TLC, HPLC and/or GC. The performance parameters presented below compare the performance of the Triage® device with other confirmatory techniques.

Analyte	Triage/Conf. Sensitivity(%)	Triage/Conf. Specificity(%)	% Agreement With Other Techniques
TCA	202/206 (98%)	184/185 (99%)	386/391 (99%)

The precision at the cut-off revealed that a specimen containing 1000 ng/mL will produce a positive result 97.5 ± 2.1% of the time. Resolution of the discrepant results yielded a sensitivity of 99 ± 1.2% and specificity of 99 ± 0.8%. The clinical performance, speed, and additional performance characteristics will be presented. The Triage test is a rapid alternative or supplement to instrument based drug screening tests. This test affords its use in emergency room situations.

Key Words: Tricyclic Antidepressants, Urine Screening Test, Immunoassay.

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Abuscreen OnLine® Immunoassays for the Detection of Drugs of Abuse Testing in Urine on the Hitachi 911, 737 and 747

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Applications for the Abuscreen OnLine 1000 test kit for amphetamines, barbiturates, cocaine metabolite, benzodiazepines, methadone, opiate, PCP and THC have been developed for the Hitachi 911, 737 and 747.

The assay system is based on the Kinetic Interaction of Microparticles in a Solution (KIMS) where microparticle aggregation is inversely proportional to the analyte concentration in urine. No pretreatment of samples is necessary.

For the Hitachi 911 and 747 four-point standard curves are generated from 0 to 2.0X ng/µL using four parameter logit/log curve fit for all assays, except THC, where a five-point, five parameter logit/log curve is generated. Typical assay precision at the cutoff is < 6%.

Additional applications for the Hitachi 911, 373 and 747 using single point calibration with the cutoff calibrator set to "zero" were generated. A positive result is reflected in any test result that is greater than zero. In all assay applications for the recommended cutoffs, a > 95% confidence level of the 0.8X being negative and the 1.2X being positive was observed. Good clinical correlation was also observed when compared with GC/MS confirmed samples.

Abuscreen OnLine has been applied to the Hitachi series of instruments. These assays have good precision and are accurate for the detection of amphetamines, barbiturates, cocaine metabolite, benzodiazepines, methadone, opiate, PCP and THC in human urine.

Key Words: OnLine, Hitachi Instruments, Drugs of Abuse.

116A

A Convenient and Sensitive Determination of Morphine and Codeine in Blood, Liver, and Urine Using Deuterated Internal Standards, Solid-Phase Cartridge Extraction and Mass-Selective Detection

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A rapid, sensitive and specific method for the quantitation of free morphine and codeine in haemolysed whole blood, enzyme-digested liver, and urine is described.

Deuterated internal standards were added to the fluids which were then extracted on mixed-mode cation exchange/reverse phase extraction cartridges. Recovered extracts were derivatized with pentafluoropropionic anhydride for analysis by gas chromatography/mass-spectroscopy/electron impact ionization. Two ion pairs were monitored for each drug.

Recoveries, with coefficients of variation, of morphine and codeine from tissues spiked at levels ranging from therapeutic to toxic are reported.

Key Words: Morphine and Codeine, Solid-Phase Extraction, Deuterated Internal Standards.

116B

Emit Analysis of Whole Blood: An Automated, Sensitive and Cost-Effective Method Using Modified Reagents for the Analysis of 0.25 mL Blood with 17 Emit Assays

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This report describes an improved method for the analysis of whole blood (0.25 mL) using 17 different Emit assays. The method is simple, rapid and automated. Alteration of Emit assay kinetics by a commercially available product (Raichem) allows for increased sensitivity and cost-effectiveness. Whole blood (0.25 mL) is mixed with acetone and the resultant supernatant is analyzed directly using a Cobas-Bio analyzer. Sample preparation is simple, rapid (4 min.) and requires no filtration or evaporation steps. Limits of detection are typically 10 fold lower than the Syva low calibrator concentrations, allowing detection of therapeutic or sub-therapeutic drugs in all 17 assays. The number of assays achieved per kit is increased to 800 or 1600. The Emit assays used include; amphetamine, barbiturate, cocaine metabolite, methadone, opiate, tricyclic antidepressant, benzodiazepine, methaqualone, phencyclidine, propoxyphene, cannabinoid, acetaminophen, carbamazepine, valproic acid, theophylline, lidocaine and phenytoin. The within-run CV is between 1 and 2%. The proposed method can also be used to analyze packed cells, bile, vitreous humor, saliva and tissue homogenates. The instrumental method can easily be adapted to other analyzers.

Keywords: Emit, Blood, Drugs.

volume blood + 2 vols

acetone

116C

Acetylsalicylic Acid - A Useful Reagent in Forensic Toxicology

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Salicylic acid- and acetic acid esters are produced, when acetylsalicylic acid-containing solutions react with hydroxy compounds. If this reaction is not taken into account during the analysis of biological fluids and tissues, it may lead to difficulties in the interpretation of GLC and GLC/MSD findings if the reaction products are not included in the available libraries. Alternatively, this reaction can be used to identify numerous hydroxy compounds.

The retention indices of about 80 salicylic acid esters of common hydroxy compounds were determined. Possible applications for this reaction include the analysis of congener and putrefaction alcohols in biological material which may supplement and extend the standard head-space methods already in use; and in the simple identification of traces of alcohols in pure "alcoholfree" solvents (e.g., ethanol in chloroform).

The procedure can be used for the transfer of the acetyl group in the overhead technique for the detection of weakly volatile compounds (e.g., morphine - monacetyl - diacetylmorphine), thus offering a less aggressive alternative to the acetic anhydride method.

Key Words: Acetylsalicylic Acid, Over-Head Esterification, Congener Alcohols.

117

Identification of Cocaine in Hair of Children Living with Adult Users

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Patients in this study were participating in substance abuse treatment for cocaine dependence. Head hairs from 18 patients and 38 household members (including 36 children) were analyzed. Cocaine was identified in the hair test aliquots (20 mg) of 80 percent of the adults and 78% of the children. Of households where an adult's hair contained cocaine, 86% included at least one child whose hair was cocaine positive. In some cases, the children's hair samples contained more cocaine than the adult user. Benzoylecgonine was also detected in hair from users and household members. Saliva from virtually all children tested negative, providing no evidence that children ingested cocaine. If one assumes that young children are not intentional cocaine users, these results show that their hair can become cocaine positive through environmental exposure when they live with a cocaine user.

Key Words: Hair, Cocaine, Drug Testing.

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Effect of Shampoo on Cocaine Uptake in Hair

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This study was designed to test whether shampoo affects the affinity of hair for externally applied cocaine. Cocaine-negative hair, cut from a Black, an Asian, and a Caucasian, was washed in 12 commercially available shampoos. Aliquots (20 mg hair) were soaked eight hours in 1 μg cocaine/l mL 0.1 M phosphate buffer, pH 6.0, followed by one 30 min methanol wash and three 30 min phosphate buffer washes. GC/MS quantitation revealed cocaine in all of the soaked hair samples. Concentrations depended on the shampoo used and ranged from 3.1 - 8.8 ng/mg Caucasian hair, 5.3 - 9.6 ng/mg Asian hair and 5.5 - 15.5 ng/mg Black hair. Pert® (Oily and Fine) and Prell® (Normal and Normal/Oily) washing preceded some of the highest results while Head and Shoulders® (Fine/Oily), Neutrogena® and Rave® washing preceded some of the lowest cocaine concentrations in hair. In each of the 12 shampoo experiments, the relative concentrations of cocaine in hair among the three individuals were Caucasian < Asian < Black.

Key Words: Hair, Cocaine, Drug Testing.

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Influence of Tolfenamic Acid on Metal Ion Distribution in Guinea Pig Tissues

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Tolfenamic acid is a NSAID potentially capable of forming complexes with metals in vitro. The effect of the administration of tolfenamic acid on the zinc, copper, magnesium and calcium concentrations in guinea pig tissues was studied. Tolfenamic acid, 8.5 mg/kg/day, was incorporated into the diet of adult female guinea pigs for eight weeks. Samples were collected, wet digested and analysed. Urine was collected once a week.

Thigh bone and whole blood zinc concentrations significantly decreased while spleen, uterus and adrenals zinc concentrations increased. Ovary and whole blood copper concentrations were significantly decreased while significant increases were observed in spleen, thigh bone and adrenals. Calcium concentrations were significantly decreased in kidneys, ovaries and lungs. Spleen, ovaries and thigh bone magnesium concentrations were significantly increased. Four weeks after the beginning of the administration of tolfenamic acid, we observed that excretion in urine of all the metals studied had increased.

These results support our opinion that drugs capable of forming complex compounds with metal ions in vitro could cause translocation and/or elimination of these ions in vivo.

Key Words: Tolfenamic Acid, Metal Ion, Metal Complex.

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The Use of Responsibility Analysis to Determine the Contribution of Drugs to Accident Causation

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The contribution of drugs other than alcohol to motor vehicle accident causation has been the subject of much debate. Whilst many of the so called psychoactive drugs have the potential to impair psychomotor activity, assessment of actual risk in driving has been little studied. In order to assess a possible risk for these drugs in fatal accidents, a retrospective responsibility analysis of 1045 fatal accidents was conducted in order to ascertain whether drugs are over-represented in drivers deemed responsible for the accident.

The responsibility analysis was based on that published by Robertson and Drummer (Accid. Anal. & Prev., 1994; 26: 243-47) and involved an assessment of relevant factors critical in determining responsibility. Each factor was rated numerically by the use of strict scoring guidelines to minimize subjectivity. Drivers were subsequently rated either as culpable, contributory or not culpable. Statistical analysis was conducted by logistic regression with adjustment for age and sex.

Cannabis was the most common drug detected in drivers killed at 11%, followed by amphetamine-like stimulants (3.7%), benzodiazepines (3.1%) and opiates (2.7%). Stimulants were mainly represented by methamphetamine, ephedrine, phentermine and pseudoephedrine. Benzodiazepines were most commonly diazepam, temazepam, oxazepam and nitrazepam. Opiates were predominately methadone, morphine and codeine. Other drugs accounted for a further 5.6%. Cocaine was involved in only 1 case while alcohol (>0.01 gram/100mL) was detected in 36% of the drivers.

Responsibility analysis of only those drivers in whom one drug was detected showed that the relative risk (relative to the drug-free drivers and corrected for age and sex) was 6.8 (3.9-12) for alcohol ($p<0.001$), 2.6 (0.6-12) for opiates, 1.8 (0.6-5.4) for stimulants, 1.7 (0.4-8.1) for benzodiazepines and 0.6 (0.3-1.2) for cannabis. Confidence intervals (95%) are shown in parentheses.

Key Words: Performance Impairment, Drugs, Accidents, Responsibility Analysis.

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The State of Colorado's Drug Recognition Expert (DRE) Program: They Are Not Thumbing Their Noses Anymore

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For law enforcement, the scenario is familiar and frustrating: a driver is stopped on suspicion of alcohol impaired driving and performs poorly on the roadside sobriety tests, only to "pass" a breath alcohol test. Is the driver ill; injured? Or impaired by a drug other than alcohol? Recognizing the increasing prevalence of this scenario, the Los Angeles Police Department spearheaded a procedure that a trained police officer could perform in order to obtain compelling evidence establishing that a suspect was impaired at the time of the stop. The National Highway Traffic Safety Administration conducted several laboratory and field studies which validated that a DRE officer could predict the presence of certain drug categories in the majority of cases.

In 1987, Colorado became the third state to implement a DRE program within its borders. Since the inception of the Colorado program, over 120 police officers have been certified in this procedure resulting in an increase in the identification of drug impaired drivers. One agency, the Denver Police Department, went from nine drug impaired arrests in 1987 to 149 in 1989. As with any new program, challenges have been met along the way. Such as the increased cost of toxicology tests, increased processing time and prosecutors who are unfamiliar with the DRE program. While the police community has supported the program, support from some prosecutors and courts could be improved. A correlation of DRE evaluations versus toxicology results was evaluated along with the prevalence and type of drugs found.

Key Words: Drug Recognition Expert, DRE, Drug Impaired Driving.

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Driving Under the Influence of Drugs Other Than Alcohol: Is Norway a Special Case?

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The frequency of drugged drivers apprehended in Norway has been compared to other countries. The frequency appeared to be several times higher in Norway than in any other country. Many countries do not carry out regular control and registration of drugged driving. For countries performing some sort of routine control, benzodiazepines (BZD) and tetrahydrocannabinol (THC) are two of the most frequently detected drugs. There has been a marked increase in the number of Norwegian drivers suspected of driving under the influence of drugs other than alcohol. In 1983, such cases represented about 8% (n=900) of the total number of drivers suspected of driving under the influence, increasing to 35% (n=2966) in 1993. Besides THC, the most frequently detected drugs are BZD (35%), often in supratherapeutic concentrations, combined with alcohol and/or other drugs. The number of amphetamine positive cases has increased more than 100% during the last two years (n=216 and n=476, respectively).

The main reasons for apprehending individuals include road traffic accidents, irregular or dangerous driving or some sort of suspicion of the police. The prevalence of BZD, THC and amphetamine in blood samples from drivers involved in car accidents, indicated increased accident risk to be associated with the use of high doses of BZD, cannabis and amphetamine. The frequencies of alcohol and drugs in fatally injured Norwegian drivers (n=159) were 28.3% and 16,4%, respectively. BZD and THC were the drugs most frequently found. The prevalence of drugs other than alcohol in fatal crashes in Norway was similar to other countries, indicating that the difference in the prevalence observed for living apprehended drivers might be due to national differences in the detection of drugged drivers not involved in fatal accidents.

Key Words: Drugs, Driving, Accidents.

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Psychomotor and Cognitive Measurements of Alcohol Impairment

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Thirty-four (34) unpaid, non-alcoholic volunteers (26 males, 8 females/ av. age - 40 yrs.) participated in two identical experiments in which sober performance was compared to scores obtained with moderately elevated blood alcohol concentrations (mean BAC 68 mg/dL). Two standardized microprocessor tasks, one designed to measure psychomotor performance and the second to evaluate cognitive abilities, were used to evaluate each participant. Individual alcohol free scores were compared to alcohol scores on a group and individual basis. Careful examination revealed that results of the first session were of limited value since in many instances a stable baseline had not been established. Therefore, only the results of session two were considered in this analysis. One hallmark of alcohol's actions was that it normally compounded the variability of any specific measurement of performance when compared with the sober condition. The effect of alcohol on a given subject's performance proved difficult to predict at the lower BACs obtained in this study. Certain individuals exhibited gross deterioration after alcohol administration while others improved their score. Variation in alcohol free ability compounded the difficulty. However, psychomotor performance proved to be more sensitive to alcohol than most cognitive measurements. The quantified measurement of performance provided a useful means to measure and compare performance deficits caused by alcohol.

Key Words: Cognitive, Psychomotor, Impairment.

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Testing Human Fluids for Amatoxins by RIA and LC/MS

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Although only about 2% of mushroom species are poisonous, serious poisonings continue to occur due to the inability to distinguish toxic from non-toxic mushrooms. *Amanita phalloides* causes more than 50% of all mushroom poisonings and the *Amanita* genus accounts for 95% of related fatalities.

In France, only the toxicological laboratory of the Institute of Legal Medicine of Strasbourg performs analysis of amatoxins. In 1993, 15 cases of poisoning were recorded, involving *Amanita phalloides*, *Lepiota bunneo incarnata* and *Lepiota helveola*. All the patients recovered. The screening procedure was performed by RIA (Buhlmann Laboratories) of urine samples. Amatoxins were detected for four days in most cases, with concentrations ranging from 1 to 104 µg/l. Using the same method, the time course in feces was monitored, indicating fecal elimination of amatoxins. Thromboplastin time and hepatic aminotransferases changes were evaluated in all cases.

Particular attention was focused on a poisoning case involving *Lepiota bunneo incarnata* in a 40 year old man. At admission, 24 hours after intake, AST and ALT were 10800 and 13300 IU, respectively, with a thromboplastin time of 24%. Maximal decrease of the factor V was observed 48 hours after intake. Amatoxins were detected in urine (1-70 µg/l) and feces (8-221 µg/l) for 96 and 84 hours, respectively.

A confirmatory method for alpha-amanitin and beta-amanitin in blood was established by LC/MS on a API-I Perkin Elmer Sciex, using a C18 Microbore 250 mm x 1.0 mm column. Ions monitored (M + H) were the molecular ions, 919 and 920 for alpha-amanitin and beta-amanitin, respectively. In 1994, LC/MS, because of its sensitivity and specificity, will be the technique of choice for measuring amatoxins in clinical and forensic laboratories.

Key Words: Amatoxins, RIA, LC/MS.

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Pharmacokinetic Profiles of Intravenous, Intranasal and Smoked Cocaine

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The route of administration of cocaine can substantially alter its pharmacokinetic profile. When cocaine base is smoked (SM), there is a bolus of concentrated vapor delivered to the lungs which results in rapid absorption into the general circulation in a manner similar to that observed following intravenous (IV) administration. Intranasal (IN) cocaine is absorbed through the mucous membranes of the nose in a slower manner. Consequently, blood levels are not expected to reach equivalent concentrations to those produced by the IV route. We administered cocaine to 6 male volunteer subjects who had a history of recent cocaine use and who provided informed consent to participate in the study. Doses (placebo; IV, 25 mg; SM, 42 mg; IN, 32 mg) were administered in randomized order under double-blind conditions. Blood samples were analyzed by gas chromatography/mass spectrometry with deuterated internal standards. Each route provided different pharmacokinetic profiles. Mean peak concentrations (ng/mL) and mean peak times (hrs) for cocaine and benzoylecgonine are illustrated in the table.

		Cocaine			Benzoylecgonine		
Route	N	Peak ng/mL	Peak hrs	AUC ng-hr/mL	Peak ng/mL	Peak hrs	AUC ng-hr/mL
IV	6	225	0.04	256	110	2.3	769
SM	5	206	0.04	334	85	1.4	682
IN	6	65	0.88	325	135	2.7	1071

Key Words: Cocaine, Pharmacokinetics, Route of Administration.

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The Effect of Tranylcypromine on Platelet MAO-B Activity

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A method was developed to determine platelet monoamine oxidase B (MAO-B) activity due to the difficulties encountered in the measurement of irreversible monoamine oxidase inhibitors (MAOIs). Platelets were isolated from EDTA blood using existing methodology and platelet MAO-B activity was determined after incubation with benzylamine, a MAO-B specific substrate. The reaction was stopped by the addition of 1M sodium hydroxide, benzylamine was extracted into toluene and concentrations were determined with gas chromatography with nitrogen phosphorus detection. The rate of reaction was expressed as the rate of disappearance of benzylamine in micromoles per minute per milligram of protein.

A healthy volunteer took a 10 mg single oral dose of tranylcypromine, an irreversible MAOI. MAO-B activities and tranylcypromine concentrations were determined at timed intervals. Blood tranylcypromine concentration peaked at 52.8 µg/L after 1 hour 15 minutes and was not detectable at 2 hours, the limit of detection was 5 µg/L. Platelet MAO-B activity started to decrease after 30 minutes, was still depressed at 24 hours and had not returned to normal by 4 days. The MAO-B activities from 18 healthy volunteers were determined and these ranged between 0.75 and 1.45 µM/min/mg.

Determination of platelet MAO-B activity may be a more reliable and cost effective method for the detection of MAOI use as opposed to the measurement of individual drug concentrations.

Key Words: Monoamine Oxidase, Monoamine Oxidase Inhibitors, Tranylcypromine.

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Blood Concentrations and Pharmacological Effects After Oral Heroin Administration

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The oral route of self-administration of heroin is uncommon in drug users. Hydrolysis in the digestive tract and loss due to first pass metabolism results in ineffective and slow delivery of drug to the brain. However, heroin may be ingested accidentally, or deliberately in an effort to prevent discovery of drug possession or recovery of evidence. Little is known about the resultant blood concentrations of heroin and metabolites and any subsequent pharmacological effects. Published data are limited to reports of individual overdose cases of 'body packers'. Therefore, we conducted a controlled clinical study in which six healthy male volunteers with a history of heroin use were orally administered 10 mg of heroin hydrochloride. Blood samples, physiological and subjective measures were collected at specific timepoints following drug administration. Blood samples were analyzed by solid phase extraction-gas chromatography/mass spectrometry for heroin, 6-acetylmorphine and morphine. Heroin and 6-acetymorphine were not detected in blood after ingestion. Morphine was first detected 7.5-45 min after drug administration. Peak morphine concentrations ranging from 2.2-15 ng/mL (mean = 8 ng/mL, N=6) were achieved 7.5 min to 4 h after drug administration. Thereafter, morphine concentrations declined, reaching the limit of sensitivity of the assay (1 ng/mL) by 1-12 h. Measurement of pupil diameter indicated a mean change from baseline of < 1.0 mm. 'Feel' drug and drug 'Liking' scores were variable, with 3 subjects reporting no effect at all and 3 reporting a slight or moderate 'good' effect 15-60 min after ingestion. These data indicate that heroin produces low blood concentrations of morphine and weak pharmacological effects following low dose oral administration.

Key Words: Heroin, Oral Administration, Blood.

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Identification of 7-Amino Benzodiazepine Metabolites in Urine Through Concurrent Use of Oxazepam and Temazepam

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Patients undergoing treatment for heroin dependence frequently consume one or more benzodiazepine tranquillisers. Some of the more potent drugs in this class are responsible for antisocial and violent behaviour in patients and jail inmates.

EMIT (Enzyme Multiplied Immunoassay Technique, Syva Co.) followed by gas chromatography-mass spectroscopy is an effective procedure for identifying individual benzodiazepines. Although EMIT is sensitive to both oxazepam and temazepam, sensitivity to the 7-amino metabolites of flunitrazepam and clonazepam are respectively, 2.5 and 50 times less than that of oxazepam. These 7-amino metabolites are often identified in urine only because of concurrent ingestion of either oxazepam or temazepam, both yielding a strong EMIT response. Using a standard 0.2 µg/mL cutoff, 66/345 positives (19.1%) contained 7-amino benzodiazepines in addition to oxazepam or temazepam. A further 56 (16.2%) contained only 7-amino metabolites.

GC/MS analysis of 93 prisoner urine samples producing an initial EMIT value 0.1-0.2 (normal cutoff, 0.2 µg/mL) showed a high proportion (50, 54%) positive for 7-amino benzodiazepine metabolites alone (concentration range 0.2-7.6 µg/mL). It may be appropriate to select a lower EMIT cutoff for this assay in order to detect these particular metabolites.

Key Words: Benzodiazepines, EMIT, Cutoff.

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Twenty-Three Percent (23%) Misdiagnosis Rate Among Cocaine Exposed Babies: A Report

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Meta-hydroxybenzoylecgonine (m-OH-BZE), a minor urinary metabolite of cocaine in adults, has recently been reported as possessing sufficient immunoreactivity to cause inconsistent data in meconium screening procedures.

To determine the significance of this report, we analyzed m-OH-BZE, benzoylecgonine (BZE) and cocaine in two hundred and eight (208) consecutive meconium samples received in our laboratory which screened positively for cocaine metabolites by fluorescence polarization immunoasssay (FPIA). Using solid-phase extraction and GC/MS, 132 (63%) were confirmed for cocaine, 161 (77%) for BZE and 197 (95%) for m-OH-BZE. Monitoring the presence of m-OH-BZE in meconium resulted in a 49% and 22% increase in confirmation rate as compared to cocaine and BZE respectively. Twenty-three percent of the samples contained m-OH-BZE only; a mere 2% contained BZE only.

We conclude that almost a quarter of cocaine exposed babies are potentially being misdiagnosed, since m-OH-BZE is not a routine analyte in commercial meconium testing laboratories.

Key words: Meconium, Meta-hydroxybenzoylecgonine, Fetal Metabolism.