ABSTRACTS

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1 GC/MS Analysis of Three in vivo Metabolites of Cocaine as Indicators of Cocaine Ingestion

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The human body metabolizes cocaine to form primarily benzoylecgonine (BZE) and ecgonine methyl ester (EME). Since the urinary elimination half-life of BZE (4.5 hrs.) is greater than EME (3. 1 hrs.), many urinalysis work-place drug testing laboratories utilize quantitative Gas Chromatography/Mass Spectrometry (GC/MS) methods that target BZE to indicate the use of cocaine. However, a positive BZE urine result is sometimes challenged in court proceedings on the pretense that the presence of BZE resulted from the addition of cocaine to the urine sample with subsequent *in vitro* hydrolysis to BZE. For this reason, a sensitive and rapid GC/MS procedure was developed for the simultaneous analysis of the following cocaine metabolites that arise exclusively via *in vivo* metabolism: m-hydroxy benzoyl ecgonine (m-OHBZE), p-hydroxy benzoyl ecgonine (p-OHBZE) and N-desmethyl benzoyl ecgonine (norBZE). The cocaine metabolites were extracted from urine after the addition of the internal standards [BZE-D3 (BZE), m-OHBZE-D3 {m-OHBZE, p-OHBZE, and norBZE)] using XTRX RP/W (Creative Technologies Systems, Inc) extraction cartridges (styrene/divinylbenzene). The dried extracts were first derivatized with propyl iodide in DMSO/TMAH/TMPH to form the propyl esters and then acetylated with acetic anhydride to form the N-Acetyl (norBZE) and O-Acetyl (m-OHBZE and p-OHBZE) products. The observed Limit of Detection (LOD) for m-OHBZE, p-OHBZE, and norBZE was 5ng/mL. Recovery, of m-OHBZE, p-OHBZE, and norBZE was 88%, 87% and 86%, respectively. Analysis of sixteen human urine specimens that had previously been found to contain BZE by Gas Chromatography/Mass Spectrometry (GC/MS) using a cutoff of 100 ng/mL, showed the presence of norBZE in all 16 specimens and m-OHBZE in 15 specimens studied. p-OHBZE was detected in only six of sixteen specimens. The simultaneous analysis of human urine for m-OHBZE, p-OHBZE, and norBZE specifically demonstrates that cocaine was ingested. Furthermore, use of these three metabolites will provide a more reliable indication of *in vivo* cocaine metabolism since they are more prevalent than EME due the potential hydrolysis of EME to ecgonine.

Key Words: cocaine, metabolism, and analysis

2 The Effect of Time. Temperature and Type of Container on Stability of THC

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The effect of time, temperature and container material on the stability of 9 tetrahydrocannabinol (THC), the active constituent of cannabis, was investigated.

Drug - free blood was spiked with THC standard (in methanol) at two concentrations - 10 ng/mL, and 50 ng/mL. The blood was aliquoted into 5 different container tubes - 1) polypropylene, 2) polystyrene, 3) polycarbonate, 4) untreated glass and 5) silanised glass, and stored at 2 different temperatures; -20 C and -60 °C. Samples were then analysed at l, 2, 3, 4 and 8 week time points using GC/MS in SIM mode.

Stability of THC was most affected by temperature rather than container type. At 8 weeks, the average THC concentration for 10 ng/mL spike samples in all tubes stored at -20 °C (n=15) was 4.21 ng/mL 0 .28, significantly lower compared to all tubes stored at -60 °C (n=18), where the average THC concentration was 8.76 ng/mL 0.17 ($p<0.001$). Analysis of the 50 ng/mL spike at 8 weeks showed comparable results, with all tubes stored at -20 °C (n=18) reporting an average THC concentration of 26.50 ng/mL 1.29, significantly lower compared to all tubes stored at -60 °C (n=18) where the average THC concentration was 44.24 ng/mL. 1.01 ($p<O.OOl$).

No significant differences were seen between the two temperature groups at the 1 and 2 week time periods, for either spike group ($p>0.05$). The trends seen in the 8 week readings became more apparent at the 3 and 4 week time points. Comparison of tube types revealed that there was no significant difference between

container types, at any time point, at either temperature, for either spike group ($p>0.05$). Investigation of the effect on samples left at room temperature will also be presented.

These findings suggest that there are important issues to consider in storage of blood samples that are analysed for THC, specifically the temperature samples are stored at and length of time before analysis.

Key words: Cannabis, stability, temperature.

3 Effect of Pyridinium Chlorochromate Adulterant (Urine Luck) on Drugs of Abuse and a Method for Quantitative Detection of Cr^{6+} in Urine.

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An oxidative adulterant known as pyridinium chlorochromate (PCC, Urine Luck) is used by drug abusers to mask the presence of drugs in urine. In a systematic study, we found that the PCC at concentrations $\leq$104 µg/ml (Cr^{6+} $\leq$ 25 µg/ml) in urine produced colors that could not be distinguished from the color of normal urine. When 11-nor-delta-9-THC-9-carboxylic acid (THC-acid) was treated with 25 µg/ml of Cr^{6+} at room temperature for 31 hr, approximately 57% of the THC-acid was oxidized and lost. In 66 hr at room temperature the drug was completely oxidized. During the process of extraction, only 23% of d_3-THC-acid (internal standard) was lost indicating the reaction started almost immediately. Codeine, morphine, PCP, benzoylecgonine, amphetamine, and methamphetamine were not effected by the Cr^{6+} under the same condition. When acid hydrolysis was performed for codeine and morphine, all morphine including the d_3-morphine (internal standard) were lost. Oxidation in presence of acid during extraction was the reason for the loss of morphine. Codeine was uneffected by Cr^{6+} /H^+.

To detect the Cr^{6+}, 2.5 ml of urine were treated with 0.5 ml of 0.5% diphenylcarbazide in acetic acid and acetone (1:9). A characteristic intense purple color indicated the presence of Cr^{6+} and was a complex formed from chromous (Cr^{2+}) ion and enol from diphenylcarbazone. The concentration of Cr^{6+} was measured from the color intensity at wavelength 544 nm using a spectrophotometer. The quantitation was linear over the concentration range 60-1200 ng/ml (PCC 250-5000 ng/ml). The intercept, slope, and correlation coefficient (r) were -0.11, 1.00, 0.9999, respectively. The limit of detection of the procedure was 30 ng/ml of Cr^{6+} . To test an urine dipstick method, strips of Thomas filter paper (4705-C-20) were soaked into 0.5% diphenylcarbazide, dipped into urine, and allowed to dry for 2 min. Purple color indicated presence of Cr^{6+}. LOD for the dipstick method was 480 ng/ml (PCC 2 µg/ml). The diphenylcarbazide method is effective for screening or quantitating Cr^{6+} , i.e. Urine Luck, in urine.

Key Words: Chromium Adulteration, Effect on Drugs, Detection

4 Deposition of [^{3}H]Cocaine, [^{3}H]Nicotine, and [^{3}H]Flunitrazepam in Mouse Hair Melanosomes after Systemic Administration

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The general acceptance of reliability of hair for drug testing is awaiting the determination of the mechanism(s) of drug deposition. Questions remain on the efficiency of drug extraction, the differentiation of drugs deposited from systemic circulation from external contamination, the role of sweat and sebum in contributing to hair drug levels and the role of pigment in drug deposition. Determining the subcellular localization of drugs deposited in hair from the systemic circulation and identifying differences in deposition between pigmented and nonpigmented hair may help to provide answers to some of these remaining questions. This study utilized three labeled drugs of forensic importance, C57 (eumelanin pigmented) and

Balb/C (nonpigmented) mice and microautoradiography to examine the subcellular distribution of drugs in developing hair.

Two C57 and two Balb/C mice (23 days old) were injected with either [benzoyl-3,4-^{3}H]cocaine, [methyl-^{3}H]flunitrazepam or [*N*-methyl-^{3}H]nicotine (New England Nuclear Corp.) at a known concentration and specific activity for each of five time points. Animals were sacrificed and skin samples were fixed in 10% buffered formalin, imbedded in paraffin, sectioned and affixed to slides. Sections were dehydrated, defatted and dried then coated in photographic emulsion (NTB2, Eastman Kodak) for autoradiography. Slides were allowed to expose for 8 weeks in light-tight boxes after which they were developed, stained with H&E and examined with a Nikon Microphot-FX fitted with a filter block for incident lighting of specimens. Silver grains were counted in follicles from both animals.

Skin sections taken at early time points (10 to 15 minutes after dosage) show rapid association of each drug with melanin within the hair bulb of C57 mice. Silver grain deposition, indicating the presence of tracer, is clearly over the eumelanin melanosomes of the forming medulla and cortex in the hair bulb without any deposition over the other cellular structures or other parts of the hair bulb. No silver deposition was evident outside the forming cortex and medulla in C57 mice. Little silver grain deposition was evident in Balb/C mice treated in the same manner as C57 mice. The appearance of tracers in sebaceous glands differed for each drug. [^{3}H]Flunitrazepam appeared in the sebaceous glands of Balb/C mice but not C57 mice while [^{3}H]cocaine appeared minimally in both Balb/C and C57 mice and [^{3}H]nicotine did not appear in the sebaceous glands of either strain. And although all three drugs are labeled in such a manner that the major metabolites are also radiolabeled, the time course of the deposition of drug into melanin, when compared with the half-lives of the drugs suggest that parent drug is predominantly deposited in the hair.

Microautoradiography was employed to show that association of drugs from the serum directly with forming hair pigment is a primary pathway of deposition into the hair. After systemic administration, all three radiolabeled drugs associated with melanin in the forming hair within minutes of dosage. Sebum was determined to be an insignificant deposition route for all three drugs.

Keywords: Deposition, Hair, Drugs

5 Sensitive Quantitation of Clonazepam and 7-Aminoclonazepam in Hair

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Clonazepam (Klonopin™, Clonex, Iktorivil, Rivotril) is an anticonvulsant benzodiazepine producing similar pharmacological effects (depression, amnesia) to other compounds from the same therapeutic class. In combination with alcohol, the CNS-depressant action can be significantly potentiated. As with other benzodiazepines, clonazepam (CLO) is a possible drug used in "date-rape"situations.

The aim of this study was to develop a sensitive, precise and accurate analytical NCI-GC-MS method for detection of CLO and its major metabolite 7-aminoclonazepam (7ACLO) in hair with potential application to alleged drug-facilitated rape victims. To determine the feasibility of detecting 7ACLO and CLO, hair samples collected from psychiatric patients treated with CLO were analyzed. Five point standard curves for 7ACLO and CLO were prepared by spiking 50 mg aliquots of pulverized drug free hair. The ranges of the standard curves were 1.0 pg/mg - 100 pg/mg for 7ACLO and 10 pg/mg - 400 pg/mg for CLO. In addition, two levels of control hairs were prepared for CLO (30 and 300 pg/mg) and 7ACLO (3 and 60 pg/mg). So far our lab has received 4 hair samples for analysis. All samples were pulverized, and internal standard, D_5 diazepam(600 pg/mg), was added to 50 mg aliquots of hair, standards and controls. Methanol (3 ml) was added and vials were sonicated for 1 h. Methanol was decanted and stored, and 0.1N HCl was added (3 ml) to hair. The specimens were incubated overnight at 55^{0}C. The fractions were combined and mixed mode solid phase extraction was performed. Drugs were eluted from the column using methylene chloride:isopropanol:NH_4OH (78:20:2, v/v/v). Extracts were evaporated to dryness, and derivatized with HFBA (50 µl). HFBA was evaporated and ethyl acetate (25 µl) was added. A Hewlett Packard GC-MS system comprising a 6890 GC and a 5973 MSD (CI with methane) was operated in SIM mode with splitless

injection. For CLO *m/z* 315 and 279, for 7ACLO *m/z* 461 and 445, and for D_5 diazepam *m/z* 289 ions were monitored. Standard curves for 7ACLO and CLO had correlation coefficients of 0.995 and 0.998, respectively.

7ACLO was present in significant quantities in three hair samples. One patient was on CLO for two weeks only (5 mg total dose). The hair sample collected at the end of the two-week period had only traces of 7ACLO. The hair sample with the highest concentration of 7ACLO was also positive for CLO.

Key words: hair analysis, NCI-GC-MS, clonazepam, 7-aminoclonazepam.

6 Methadone Analysis in Nail Clippings of Patients on a Methadone Maintenance Program

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This study offers an analytical scheme for methadone in fingernail clippings. Samples (0.79–16.33 mg) were collected from 28 consenting adults participating in a methadone maintenance program in Edinburgh, Scotland. At the time of sampling, participants provided answers to a questionnaire regarding the drugs they had used prior and during admission to the maintenance program. The nail clippings were stored in plastic bags at the time of collection and transferred to a forensic toxicology laboratory in Glasgow. There they were decontaminated by sonication in 0.1% sodium dodecyl sulphate (SDS) for 15 minutes followed by sonication in water three times for 15 minutes each and sonication in methanol three times for 15 minutes each. Whereas the resulting SDS and water washes were discarded, the methanolic washes were collected and screened for methadone by enzyme immunoassay (EIA). Once the methanolic washes had tested negative for methadone, the decontaminated nail clippings were hydrolysed in 1M NaOH. Aliquots of the hydrolysates were screened for methadone by EIA and confirmed by gas chromatography – mass spectrometry (GC-MS). The mean methadone concentration in fingernail clippings determined by EIA was 40.79 ng/mg nail. The mean methadone concentration in fingernail clippings determined by GC-MS was 46.98 ng/mg. Hydrolysates of the equivalent of 10 mg of blank nail clippings were spiked with known concentrations of methadone and analysed by the proposed procedures. In this way, extraction recoveries and limits of detection of the two techniques for methadone in the nail were calculated. Based on our results, fingernails appear to be a potentially useful biological specimen for the analysis of methadone and the monitoring of patient compliance to their methadone maintenance programs.

Keywords: Fingernail clippings, Analysis, Methadone

7 Fatal Impaired Drug Metabolism in an Institutionalized Patient

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A 31-year-old female inmate of a correctional institution was transferred to a city hospital for two relatively minor procedures: skin grafting to repair old burns to her leg, and closure of a tracheostomy. She had a history of asthma since childhood, seizures for the previous four years, and long standing psychiatric problems. The medical procedures were well tolerated, but she developed respiratory difficulties, several hours after recovery, which continued to worsen despite aggressive treatment with bronchodilators. She was conscious at the time she collapsed and died five days post-op.

The autopsy findings were essentially negative during gross and histologic examination, with the exception of histopathology consistent with the medical history of chronic asthma, but not of an acute asthmatic attack. Routine toxicology found meperidine (blood 4.9 mg/l; liver 4.6 mg/kg), normeperidine (blood 5.0 mg/l; liver 10.6 mg/kg), trimipramine (blood 2.7 mg/l; liver 20 mg/kg), desmethyltrimipramine (blood 8.1 mg/l; liver 63 mg/kg), methotrimeprazine (blood 1.03 mg/l, liver 9.6 mg/kg), paroxetine (blood 0.27 mg/l), carbamazepine

(blood 12 mg/l) and lidocaine (attempted resuscitation). The blood was labelled as "cardiac". It is acknowledged that postmortem redistribution will account for part, of the elevated blood drug concentrations. However, the elevated drug concentrations in the blood and liver, in particular for the meperidine, normeperidine and desmethyltrimipramine, strongly indicates impaired drug metabolism or possibly impaired clearance as a primary explanation.

Except for the meperidine and lidocaine, the patient had been receiving all drugs for months or years prior to death. The dosages of the trimipramine, methotrimeprazine and carbamazepine were maintained after admission to the hospital, but the paroxetine was discontinued. The patient was under 24-hour guard during the transfer and hospitalization, and did not have access to any medications. Clinical biochemistry testing indicated that her renal and hepatic function were grossly normal prior to admission to hospital. Death was attributed to the chronic accumulation of at least four of the drugs administered (meperidine, trimipramine, paroxetine and methotrimeprazine), due to drug-drug interactions, and possibly one or more genetically deficient metabolic pathways. This case also illustrates the care with which high drug concentrations must be interpreted.

Key words: Impaired metabolism; accumulation; interpretation.

8 Fatal Strychnine Poisoning: Application of GC/MS/MS and GC/NPD for Drug Confirmation and Quantitation

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Strychnine is an infrequent human intoxicant that prevents the binding of glycine at inhibitory synapses and may produce severe convulsive activity and death. The objective of this report is to communicate toxicologic findings and application of chromatographic techniques in a human fatality involving suicidal ingestion of a strychnine containing drink. Drink contents and alkaline organic extracts of post-mortem blood and tissue tested positive by the Mandelin's color test, and qualitative analysis by GC/NPD revealed the presence of strychnine. Confirmatory testing for strychnine was performed by ion trap GC/MS/MS since structural-fragmentation information is inadequate by GC/MS analysis alone. The molecular ion of strychnine (m/z 334) was initially generated by MS-EI with subsequent production of ion fragments by collision induced dissociation under resonant waveform conditions. GC/MS/MS analysis produced a mass spectrum with ion fragment intensities at m/z 319, 306, 277, 261, 246, 233 and 220. Further GC/MS/MS/MS analysis was used to evaluate the fragmentation pathways for ions. Analysis revealed pathways for ions with m/z and product ion (m/z) of 277 (246), 306 (261, 220), 261 (233). Ions with m/z of 319, 246, 220 and 233 failed to show further fragmentation products under ion trap conditions.

Quantitation was accomplished by GC/NPD on a (5% phenyl)-methylpolysiloxane capillary column using temperature programming (100-300°C, 25°C/min). Analysis of samples prepared by alkaline n-butyl chloride extraction, acid back-extraction and a final alkaline extraction with methapyrilene as internal standard resulted in matrix-dependent variability in extraction efficiency of strychnine. A standard addition method of calibration allowed a valid quantitation (mg/L or mg/Kg) of strychnine in postmortem subclavian blood (1.82), inferior vena cava blood (3.32), bile (11.4), liver (98.6), lung (12.3), spleen (11.8), brain (2.42) and skeletal muscle (2.32) specimens from the case. Chromatographic analysis of fluid and tissue did not reveal metabolic products of strychnine in the body. The case demonstrates analytical challenges of postmortem matrices and the application of ion trap GC/MS/MS in medical examiner case work.

Key Words: Strychnine, Human fatality, Ion trap GC/MS/MS

9 Toxicology of heroin related deaths in Victoria-Australia

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The number of deaths attributed to the intravenous use of heroin has increased dramatically in Victoria in recent years. Last year (1998) there were 168 fatalities resulting from heroin use (60 deaths per 1 million persons). This represents a 60% increase from the previous year and a 5-fold increase since 1990. Heroin related deaths now represent 50% of all drug-related deaths and 12.5 % of all coronial cases.

The mean femoral blood concentration of total morphine in 1096 heroin-related deaths was 0.51 mg/L, ranging from 0.01 mg/l - 80 mglL The median concentration was 0.31 mg/L. While the purity of street heroin has increased steadily over the last 9 years, the mean concentration of total morphine in these cases has remained static with no significant change. Drugs other than morphine were detected in 85% of cases and 16% of deaths showed little or no morphine in urine.

Alcohol was detected in 36% cases with an average blood alcohol concentration (BAC) of 0.12 g/dl. Cases with alcohol had significantly lower concentrations of total morphine ($p<0.001$) than cases with no alcohol (0.35 mg/L vs 0.68 mg/L). This finding was also consistent with cases which had BAC > 0.2 g/100 mL compared with cases which had a BAC < 0.2 g/100mL.

Benzodiazepines were detected in 51 % of all cases. The frequent detection of diazepam, temazepam and oxazepam supports the notion that the consumption of benzodiazepines is an associated risk factor for heroin deaths, although concentrations of total morphine were similar in the absence or presence of benzodiazepines. The high frequency of benzodiazepines also suggests that heroin-users are regularly using those drugs to minimise withdrawal symptoms. Other drug groups also prevalent include cannabis (26%), amphetamines (11%) and other opioids (10%) Since the contribution of other drugs to death in heroin users in Victoria has remained relatively constant over the last 9 years, this does not explain the increased death rate. However, it is not clear at this stage if larger amounts of these drugs are now taken compared to previous years. A summary of the toxicological findings from 1096 heroin-related deaths will be presented.

Key Words: Heroin, death, toxicology

10 Case Study: Duragesic ® Patch Postmortem Tissue Distribution of Fentanyl in Twenty-five Fatalities

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Fentanyl is a potent, short acting narcotic analgesic widely used as a surgical anesthetic and for the control of pain when administered in the form of a transdermal patch. The success of the patch can be attributed to Fentanyl's low molecular weight and its highly lipophilic nature, which enables it to be readily absorbed through the skin and subsequently distributed throughout the body. Over the past three years, the Los Angeles County Coroner's Toxicology Laboratory has encountered twenty-five fatalities involving Duragesic® Patches (Fentanyl) and the postmortem tissue distributions are presented here.

The analysis of Fentanyl from postmortem specimens (3 milliliters or grams sample size) consisted of a Butylchloride basic extraction followed by identification and quantitation on a gas chromatograph-mass spectrometer using the selected ion monitoring (SIM) mode. The Fentanyl ions monitored were **245**, 146, and 189 m/Z and the internal standard, D5-Fentanyl ions, were **250**, 151, and 194 m/Z (quantitation ion in **bold**). The linear range of the assay was 1.66 ug/L to 500 ug/L with the limit of quantitation and detection of 1.66 ug/L.

The postmortem tissue distribution ranges of Fentanyl in the twenty-five fatalities are represented in the following table:

Modes of Death (Cases)	Fentanyl Tissue Distribution Ranges (Cases) : ug/L or ug/kg Heart Blood	Femoral Blood	Vitreous	Liver	Bile	Gastric Urine	(Total)	Spleen	Kidney	Lung
Accident (14)	3.5-139 (13)	4.5-43 (9)	8.0-20 (20)	5.8-353 (12)	3.5-197 (9)	2.9-895 (11)	1.6-1,200 (9)	79 (1)	----	31 (1)
Natural (5)	1.8-81 (5)	3.1-24 (2)	<2.0 (1)	7.1-613 (4)	7.5-262 (3)	14-113 (4)	0.01-91 (3)	----	----	----
Suicide (3)	6.3-29 (2)	6.5 (1)	------	13-204) (3)	25 (1)	18 (1)	0-7.9 (2)	----	----	----
Undeterm. (2)	<2.0-2.6 (2)	------	----	7.7-11 (2)	5.9 (1)	4.9-32 (2)	0.32-0.60 (2)	7.8 (1)	11 (1)	----
Deferred (1)	4.8	6.8 (1)	10 (1)	25 (1)	22 (1)	12 (1)	13 (1)	----	----	----

The age of the decedents in this study ranged from 19-84, with an average age of 46.
The main objectives of this paper are to show the prevalence of Fentanyl Patches in our community and to aid the forensic toxicologist with the interpretation of postmortem levels difficult cases.

Keywords: Duragesic ®Patch, Fentanyl, Fatalities

11 Propafenone Distribution in a Medical Examiner Case

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Propafenone (Rythmol) is a class I_C antiarrhythmic agent that is similar in structure to beta-blocking agents but exhibits only moderate beta-blocking and sodium-channel blocking effects. The drug was detected in a thirty-two year-old Hispanic male who was found dead. The deceased was discovered lying on a hotel bathroom floor subsequent to a welfare check by the hotel manager.

Investigation at the scene revealed cocaine under the mattress, two empty beer cans, and a bottle of prescription medication Rythmal (150 mg) containing 11 pills. Further investigation determined that the prescription was for 100 pills. At autopsy no anatomic cause of death was identified. Routine toxicology tests for alcohol and therapeutic plus abused drugs were performed on postmortem specimens. Considering information from the scene investigation, specimens were also tested for propafenone.

The drug was analyzed using a method modified from Harapat et al J. Chrom. 230:448-453, 1982.) Propafenone was separated by gradient reverse-phase high-performance liquid-chromatography using propranolol as an internal standard. The results of the quantitation of propafenone in blood, stomach content, and liver are presented below. Other drugs identified in this case were ethanol, cocaine, cocaethylene, and benzoylecgonine.

	Alcohol	**Propafenone**	**Cocaine**	**Cocaethylene**	**Benzoylecgonine**
Blood	0.20 g/dL	9.1 mg/L	< 0.1 mg/L	< 0.1 mg/L	0.65 mg/L
Stomach content		135 mg/total content			
Liver		230 mg/Kg			
Vitreous Humor	0.25 g/dL				
Urine	0.28g/dL	present			

The therapeutic level of propafenone has been reported to be approximately 2 mg/L. The presented case appears to be an overdose although at this time, further investigation is pending. Reports of the quantitation of propafenone in postmortem specimens are limited. To our knowledge, this is the first such report within the United States.

Key Words: Propafenone, Redistribution

12 The Changing Pattern of Drug Deaths in San Francisco

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During 1998, important changes occurred in the epidemiology of drug related deaths in San Francisco. Morphine (MS), cocaine (C), and methamphetamine (Meth) were detected in 257 decedents. In 66 cases (26%), the drugs were an incidental finding, unrelated to the cause of death. Mean age was 40 years, and 84 % were males (217/257). C was detected in 154 cases, MS in 150, and Meth in 44. In spite of the intense publicity accorded to proliferating Meth labs, Meth-related death totals increased insignificantly from 1997, and were fewer in number than in the mid-1990s. However, major increases were observed in the total number of heroin- related deaths and in the tendency to polypharmacy ("speedballing"). In exactly half the Meth cases (22/44), and nearly half the C cases (73/154, 47%), morphine was also detected in the blood often in high concentrations (mean = 290 ng/ml , median = 160 ng/ml; and 349 ng/ml and 190 ng/ml respectively). In cases where only MS was detected in the blood (n = 81), the median (140 ng/ml) and mean (308 ng/ml) concentrations were similar to those measured in the stimulant abusers. Prior to 1998, morphine was detected in less than 25% of stimulant-related deaths. These findings suggest (1) death from acute methamphetamine toxicity is a relatively uncommon event, (2) that the number of meth-related deaths is not increasing, (3) that heroin is much more readily available than in the past, and (4) the number of heroin-related deaths is accelerating.

Key words: methamphetamine, heroin, death

13 Lamotrigine Distribution in Two Postmortem Cases

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Lamotrigine (Lamictal), is a new anticonvulsant drug recently approved for use in the United States. It is structurally unrelated to classical anticonvulsant drugs such as phenobarbital, phenytoin and carbamazepine. Lamotrigine is well absorbed after oral administration, with bioavailability of 98%. The pharmacokinetics of lamotrigine conforms to a one-compartment model with first order kinetics. Lamotrigine is well distributed throughout the body. The volume of distribution is 1.2 L/kg. Although a therapeutic range for lamotrigine has not been definitively established, a range between 2 and 14 mg/L has been reported. Lamotrigine is extensively metabolized to glucuronide conjugates. The major urinary metabolite is the 2-N-glucuronide; other minor metabolites include the 5-N-glucuronide and the 2-N-methyl compound. Approximately 8% of the dose is excreted unchanged and 63% is excreted as conjugated metabolites in the urine.

The most common side effect of lamotrigine is the development of a skin rash that occurs at higher doses. Other side effects include ataxia, diplopia, dizziness, drowsiness, fatigue, headache, nausea, tremor and vomiting.

Two cases are presented in which lamotrigine was identified in cases investigated by the Office of the Chief Medical Examiner, State of Maryland. Lamotrigine was identified by gas chromatography-nitrogen-phosphorus detection (GC-NPD) following an alkaline extraction. A DB-5 column provided analytical separation; no derivatization was required. The drug elutes near diazepam and hydrocodone. Confirmation was achieved by full scan electron impact gas chromatography/mass spectrometry. The mass spectrum has a base peak of m/z=185, with prominent ions at m/z=255, the molecular ion, 123 and 157. Lamotrigine was quantitated in all specimens received by GC-NPD using a 4-point calibration curve, with appropriate specimen dilutions for quantitation within the standard curve.

In Case 1, primidone (11 mg/L) and phenobarbital (5.5 mg/L) were found in the heart blood in addition to lamotrigine (8.3 mg/L); in Case 2, no drugs other than lamotrigine (52 mg/L) were detected in the heart blood. The peripheral blood lamotrigine concentration in Case 2 was 54 mg/L. The liver lamotrigine concentrations in the two cases were 41 and 220 mg/kg, respectively. The medical examiner ruled that the cause of death in Case 1 was seizure disorder and the manner of death was natural. In Case 2, the medical

examiner ruled that the cause of death was lamotrigine intoxication and the manner of death was undetermined.

Key words: lamotrigine, intoxication, seizures

14 Evaluation of 15 Non-Instrumented Drug Testing Devices, II

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Interest in conducting drug tests on-site using non-instrumented drug test (NIDT) devices has continued to Bx–re [illegible] devices eQVBx–;C% O 9Bx– , UBx– Bx– O Qx– , =Bx– continuing effort to evaluate the performance characteristics of many of the newly available devices for possible use in federally-regulated and non-regulated workplace drug testing programs.

Fifteen commercial NIDT devices were tested with a reference instrumented system, a Syva ETS using Emit d.a.u. reagents. The devices included multiple and single test formats in cup, dipstick and "plate" designs.

Ninety samples were selected for each of the five HHS-authorized drug classes from specimens received at the contract laboratory serving the U.S. Federal Probation and Pretrial Services. In order to challenge the devices on their accuracy around the cutoff, based on the initial laboratory screening results, 500 samples were selected to provide about 15 giving negative responses, 60 between minus 25% and plus 25% of the cutoff, and 15 greater than 25% above the cutoff for each drug class. Ten known control samples were also tested. All samples were analyzed by GC/MS, and the results evaluated using criteria established by the HHS Mandatory Guidelines, using standard measures: e.g., sensitivity, specificity, and positive and negative predictive values. Significant variations were found between the various devices across each drug class and between drug classes:

Amphetamines	Cocaine	Opiates	Cannabinoids	Phencyclidine
FP 2.8%–67.6%	0.0% –53.8%	18.9%–76.0%	0.0%–63.9	8.5% – 72.9%
FN 5.3%–94.7%	3.1%–35.9%	0.0%–20.0%	1.9%–74.1%	0.0% – 38.7%

Data comparing device performance and operational characteristics will be presented. (Supported by the Division of Workplace Programs, SAMHSA, Contract No. 277-94-2027-04)

Key Words: Non-Instrumented Drug Test Devices, On-site Drug Testing, GC/MS

15 Ricin: A Case of Nonfatal Poisoning

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Ricin is a poison not commonly screened for in toxicological laboratories. It is a two chain polypeptide produced by the castor bean plant, Ricinus communis L. The entire plant is poisonous, however the seeds contain the highest concentration of ricin. The poison is active by all common routes of administration. The mechanism of action is ultimately a shut down of protein synthesis leading to cell death. Early symptoms of poisoning in humans include abdominal pain, vomiting and diarrhea. These are followed in a few days by severe dehydration, anuria, fever, thirst, headache, vascular collapse and shock. If death has not occurred in 3-5 days, the victim usually recovers.

Ricin itself is detectable in biological fluids by electrochemical luminescence. Because it is bound to and then incorporated into cells following administration, it's detection time window is limited to one or two days

post exposure. It is an extremely antigenic material even when administered orally, and that lends itself to the use of enzyme-linked immunosorbant assays (ELISA) to detect antibodies to ricin.

In this case there was an attempted poisoning of a male individual by his wife and her boyfriend. The poisoning symptoms were not severe enough to alert the victim at the time of administration. The victim subsequently became suspicious when he overheard conversations between his wife and her boyfriend where they discussed the ricin poisoning. At that time, about 5-6 weeks after the poisoning, a serum sample was drawn from the victim and analyzed for the presence of ricin and it's antibodies. Both IgG and IgM antibodies were detected in the serum of the victim by ELISA. The IgG was determined by direct assay, and the IgM was assayed by a capture assay. The exposure to ricin was confirmed.

Keywords: ricin, antibody, ELISA

16 Analysis of urines of "Ecstasy" users by immunological and chromatographic techniques

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The analytical monitoring of tablets sold as "Ecstasy" at all-night techno dance parties ("raves") has shown the increasing presence of amphetamine and methamphetamine besides MDMA or related methylenedioxy amphetamines (MDEA, MBDB etc.). The aim of the present study was to confirm these findings by urine analyses.

In December 1997 and August 1998 urine samples were collected anonymously from 72 "Ecstasy" users at two major rave events in Zurich. The time of collection was 1 to 8 h after the consumption. Four commercial instrumental immunoassays for the screening of amphetamines (KIMS, Cedia, FPIA, EMIT) were compared concerning their efficiency in detecting the consumption of "Ecstasy" tablets. An automated chromatographic drug profiling system (Remedi) and HPLC-DAD were used for identification and quantification of the excreted amphetamine-type compounds. Other drugs of abuse (LSD, cocaine, opioids, Cannabis, benzodiazepines) were screened by Cedia. At a cutoff of 1000 ng/mL the immunoassays were able to detect "Ecstasy" consumption in 96-98% of the 57 urines tested amphetamines-positive by HPLC, with 1-2 results per assay unconfirmed negative. There was a correlation of the results of all immunassays and HPLC in 79% of the specimens. Five Remedi results stayed unconfirmed positive. The HPLC-DAD analysis showed that 65% of the urines contained MDMA. In 15 % of the samples MDMA was present alone (0.11-39.8 microgram/mL),whereas in 28 and 22% of the samples MDMA was accompanied by other 3,4-methylenedioxy derivatives (MDA, MDEA, MBDB) and amphetamine, respectively. MDA alone was detected in 3% of the samples. In 10% of the specimens only amphetamine was present, whereas methamphetamine could be detected in 4% of the samples. 43% of the analyzed samples were Cannabis-positive, 20% positive for other drugs of abuse (cocaine, LSD etc.). Other substances often found were caffeine, ephedrine and medicaments.

Our results confirm the earlier data from the quality monitoring of "Ecstasy" tablets, i.e. the increasing appearance of amphetamine on the "Ecstasy" market. In addition they show that many ravers are multi-drug users.

KeyWords: Ecstasy, MDMA, and immunoassay, HPLC.

17 Q.E.D. Saliva Alcohol Testing in Postmortem Cases

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The disposable Q.E.D. saliva alcohol tests (Q.E.D. A150 and Q.E.D. A350) provide a very simple, fast, and reliable means for quantitative on-site alcohol detection. Saliva alcohol levels measured by Q.E.D. have demonstrated high correlation to blood levels analyzed by gas chromatography (GC) over the concentration

range of 0 - 0.35 g/dL. The purpose of this study was to determine whether or not the Q.E.D. test would be a useful tool for the determination of postmortem ethanol levels in cases where a rapid result was needed. Both saliva and vitreous specimens were used for the evaluation.

Specimens were obtained from individuals with suspected alcohol related deaths or who had a history of ethanol abuse. Oral fluid specimens were obtained pre-autopsy from the oral cavity using the Q.E.D. device and tested immediately. Vitreous specimens were obtained at autopsy in red top Vacutainer tubes and stored at 4 C until analysis. Q.E.D. results were compared with ethanol levels determined by headspace GC analysis with a Restek 5% Carbowax 20M on 60/80 CarboBlack B packed column.

Saliva Analysis. Q.E.D. tests were initially performed using the oral fluid from 50 individuals. Of these cases, 17 of the tests were valid with 8 positive. For 23 cases the oral fluid was not attainable, and for 10 cases the sample was contaminated with blood and the tests were invalid. The correlation between the oral fluid results and the blood analyzed by headspace GC was poor (r^2 = 0.8929) over the range of 0 - 0.29 g/dL.

Vitreous Analysis. Vitreous specimens were found to be the matrix of choice for analyzing postmortem cases using the Q.E.D. Only 5 of 126 specimens were found to be unsuitable and as little as 0.25 mL of vitreous humor was sufficient to obtain a result. The Q.E.D. results correlated well with the headspace GC analysis (r^2 = 0.9881). When using ethanol levels > 0.02 g/dL (n=88), an average vitreous(GC):blood ratio of 1.18 correlated well with the average Q.E.D.: blood ratio of 1.24. These results corroborate the previously reported vitreous:blood ratio of 1.12. Vitreous:blood and Q.E.D.: blood ratios of 1.33 and 1.43, respectively were determined when ethanol levels 0 - 0.35 g/dL were used (n=121).

While the Q.E.D. saliva alcohol test does not appear to be useful in determining postmortem saliva ethanol levels due mainly to the inability to obtain a sufficient or uncontaminated sample, it does provide accurate results when using postmortem vitreous humor as the testing matrix. Using an average vitreous:blood ethanol ratio of 1.2 for concentrations > 0.02 g/dL, the Q.E.D. test may be a useful tool for the estimation of postmortem blood ethanol levels in cases where a rapid result is needed.

Acknowledgment: The authors thank STC Technologies, Inc., Bethlehem, PA for providing the Q.E.D. Saliva Alcohol Tests for this project.

Keywords: Ethanol, saliva, vitreous humor

18 A Novel, Rapid And Sensitive Method For The Detection Of 5 Benzodiazepines And 6 Benzodiazepine Metabolites In Blood And Urine

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Currently available methods for the analysis of specific benzodiazepines (BZD) and metabolites in blood and urine specimens include high performance liquid chromatography (HPLC), gas chromatography (GC), HPLC-mass spectrometry (MS) and GC-MS. HPLC and GC do not provide definitive structural information and HPLC-MS instrumentation is unavailable in most forensic laboratories. Additionally, current methods for the identification of BZD by GC-MS tend to be laborious with sensitivities often exceeding therapeutic concentrations and concentrations expected in drug-facilitated sexual assault.

We present here a novel, sensitive GC-MS method for the detection of -hydroxyalprazolam, -hydroxytriazolam, alprazolam, 7–aminoflunitrazepam, 7–aminonitrazepam, lorazepam, nitrazepam, norfludiazepam, nordiazepam, oxazepam and temazepam in blood and urine. Oxazepam-d_5 was used as the internal standard. Specimens were hydrolyzed with -glucuronidase (Helix pomatia). The hydrolysates were extracted, concentrated under nitrogen and dried in a desiccator. Liquid-liquid and solid-phase extraction (SPE) procedures were evaluated to optimize recovery. Compounds were chromatographed as either unchanged drug or as the trimethylsilyl derivative using BSTFA-10%TMCS. Analysis was performed on a Hewlett-Packard (H-P) 5890 GC equipped with an H-P 5970 mass selective detector (MSD). Optimal resolution of analytes was achieved with a 100% dimethyl polysiloxane ("DB-1", "H-P1", etc.) column (15m x 0.25mm x 0.25m). The run time was shortened by 50% over previous methods by optimizing oven parameters (initial=140 C x 1 min, 25 C/min to 200 C, 15 C/min to 300 C; injector temp=255 C, detector

temp=285 C) and increasing carrier gas head pressure to 6 psi. In addition, sensitivity was enhanced by tuning the MSD to PFTBA ions >200. Ions for selected ion monitoring (SIM) were chosen based on abundance in full scan mode. Lower limits of quantitation varied for each analyte, ranging from 5-10 ng/ml.

Key Words: Benzodiazepines, Gas chromatography-mass spectrometry, Forensic analysis

19 HPLC Procedure for the Quantitation of Dextromethorphan and Dextrorphan in Urine

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Dextromethorphan (DM) is the d-isomer of 3-methoxy-N-methylmorphinan, a synthetic analogue of codeine. Although it has virtually no effect on the central nervous system, manifestations of an acute overdose, however, include hallucinations and other psychotic episodes. Due to its easy availability, DM abuse has been consistently increasing especially by high school students who seek its hallucinogenic and sedative effects.

DM is metabolized in humans almost exclusively by cytochrome P-450 2D6 enzyme to dextrorphan (DX). For this reason, DM is used as a probe drug for phenotyping individuals/populations to determine their metabolic rates.

Three procedures for the extraction of DM and DX were compared. Cation exchange solid-phase extraction (Bartoletti *et al.*, J. Pharm. Biomed. Analys, 14:1281-1286, 1996), C-18 solid-phase extraction (Ducharme *et al.* J. Chrom. B, 678:113-128, 1996) and liquid-liquid extraction (Gotschall *et al.*, submitted) utilizing Hydromatrix disposable cartridges. The latter method yielded best recoveries for both DM and DX (87 and 81 % respectively). To 2.5 mL of urine, 3300 units of -glucuronidase, and 10 ug levallorphan (internal standard) were added. After incubation, the pH was elevated and the aqueous mixture was loaded into the hydromatrix cartridge, DM and DX were eluted with hexane/butanol (9:1, v/v), back extracted, dried, reconstituted in the mobile phase, and loaded on an HPLC equipped with a fluorescent detector. Linear quantitative response curves were generated for DM and DX over a concentration range of 0.012 to 1.20 and 1.20 to 120 ug/mL, respectively. Linear regression analyses of the standard curves for DM and DX exhibited correlation coefficients ranging from 0.998 to 0.999, respectively.

The applicability of the method for the detection and quantitation of dextromethorphan and dextrorphan in approximately 100 urine samples was demonstrated successfully. Supported by grants from NIH R01-GM58647, NIH/NCRR 1P20RR11104-04, and MSM Faculty Development grant.

Key Words: Dextromethorphan, Dextrorphan, Urine

20 Development of Chromate-Detect Test for the Automated Chemistry Analyzers to Test Urine Samples Adulterated with Urine Luck

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Adulteration of urine samples to escape drug detection has become a problem in the work place urine drug testing programs. The adulterant Urine Luck is popular in certain geographical areas and is reported as next to nitrite in its use as an adulterant by drug abusers. Urine Luck is available via Internet and music shops as Tommy Chong's Urine Luck. It is manufactured by Spectrum Laboratories and is supplied as 8mL liquid in a glass vial. The directions on the package suggest adding the contents of vial to 3-5 ounces of urine and mixing slightly. The compound in Urine Luck has been identified as Pyridinium Chlorochromate at

24mg/mL, which is a very strong oxidizing agent. Urine Luck has no significant effect on the natural characteristics of urine such as pH, color, specific gravity and smell. Because of its oxidizing property, Pyridinium Chlorochromate makes most drugs undetectable by immunoassays and GC/MS.

The objective of our study was to develop a method for the automated chemistry analyzers to detect chromate in urine samples adulterated with Urine Luck. There are published reports on the determination of chromium in drinking water and wastewater samples by analytical methods such as HPLC and ion chromatography utilizing the indicator Diphenylcarbazide. The hexavalent chromium complexes with Diphenylcarbazide to produce pink color that can be measured at 540nm. Based on this principle we have developed a Chromate-Detect™ test with a ready-to-use liquid reagent and calibrators (negative and 100ug/mL chromate) for the detection of urine adulteration by Urine Luck. The within-run and between-run CVs for the low (50ug/mL) and high (200ug/mL) controls ranged from 1.7 to 4.8%. The test has linearity ranging from 5 to 1000ug/mL and Limit of Detection (LOD) 1.1ug/mL. Among the endogenous substances tested for interference, Ascorbic Acid and Creatinine showed interference by lowering the recovery of chromate by 10%. The low recovery of chromate is not a concern because in urine samples adulterated with Urine Luck the concentration of chromate is >800ug/mL well beyond the normal (0.04-1.0ug/L) and toxic (0.09-2.0ug/L) chromium levels in urine. No false positive of false negatives were detected by this test. Accuracy of the test was determined by spike recovery (94-109%) and sample correlation with the spectrophotometric and HPLC methods.

The Chromate-Detect Test is a simple, precise and reliable method, which can be applied to most automated chemistry analyzers.

KeyWords: Urine Luck, Pyridinium Chlorochromate, Diphenylcarbazide

21 Rapid Immunoaffinity Extraction of LSD from Blood and Urine.

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Extraction and detection of lysergic acid diethylamide (LSD) in biological fluids continues to be an analytical challenge due to the low dose ingested and the rapid biotransformation of the drug. High affinity antibodies raised against LSD were used to prepare a robust immunoaffinity matrix that was used to extract LSD from biological fluids in the sub-ng/mL region of forensic interest. The chromatographic support was prepared by covalently attaching anti-LSD antibodies to Protein A-coated agarose beads. $^{3}H_{3}$-LSD and radioactive counting was used to estimate the column capacity as well as the recovery of drug from blood and urine. Five milliliters of affinity matrix was sufficient to bind 12 ng LSD. The analytical recovery of LSD from whole blood (0.5 ng/mL) and urine (0.2 ng/mL) was 88 and 83% respectively. No pre-treatment of the sample was necessary. Biological sample was added to the immunoaffinity column at neutral pH. After removal of interferences with buffer, LSD was eluted from the chromatographic support using ethanol. The immunoaffinity matrix was stable to extreme pH (2-12) and was reused without any deterioration in binding capacity towards LSD (99 ± 7%, n=7).

Immunoaffinity isolation of LSD was completed in about ten minutes and offers a distinct advantage over traditional solid phase extraction techniques in terms of improved selectivity of the extract. This technique might also be useful for trace enrichment of LSD from samples that contain prohibitively low concentrations of LSD. The high recovery of drug and enhanced cleanliness of the extract improves the likelihood that low levels of LSD will be detected in samples of forensic interest.

Key Words: LSD, Immunoaffinity Extraction, and Blood.

22 EMIT II® plus THC Assay

Byung S. Moon, Kelly Cabrido, Johnny Valdez, and Kelli Ryzewski. Syva Company, a wholly owned subsidiary of Dade Behring, Inc., San Jose, CA

The EMIT II Plus THC Assay consists of liquid, ready-to-use reagents and calibrators with excellent storage and calibration stability. The reagents have shown less than 10% rate loss during nine months of storage, which should result in a minimum of 18 months refrigerated stability. On the SYVA-30R, the reagents give greater than 30 days calibration stability, at which time the study was terminated. This assay can provide qualitative or semi-quantitative result at three cutoff levels (20, 50, and 100 ng/mL). The desired cutoff level is achieved by varying the urine specimen volume (15, 9 or 5 microliters of sample, respectively) used in the assay. At least one hundred and fifty urine specimens were tested at each cutoff level on the SYVA-30R analyzer and compared to Emit II reagents and Emit 5B3 THC reagents. The results were confirmed by GC/MS. In addition, good precision, analytical recovery, and specificity were observed when tested on the SYVA-30R. Total and within run precision were determined at 5 different concentrations for each cutoff level. Total precision CVs were less than 3.5% for all concentrations for the 20 ng/mL cutoff, and less than 2.5% for the 50 and 100 ng/mL cutoff levels. Within run CVs were all less than 1.5%. Analytical recovery was tested by spiked sample analysis, and at all cutoff levels the assay showed recovery of within 80% to 120% of the nominal value. Cross reactivity to the 5 major THC metabolites was shown to be equal to or better than existing Emit THC assays. Protocols have been developed on the Hitachi* 717, and Hitachi 911 and Olympus** AU800 analyzers. The EMIT II Plus THC assay is a versatile assay designed with three cutoff levels that delivers excellent performance in both qualitative and semi-quantitative mode.

* Is a trademark of Roche Diagnostics Boehringer-Mannheim Corporation

** Is a trademark of Olympus America Inc.Keywords: THC, Cutoff Level, EMIT II Plus Assay

23 A Novel Method for Elimination of Amphetamine False Positive Results During Immunoassay Screening

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We have developed a homogeneous immunoassay method to eliminate false positive amphetamine results caused by cross-reactive substances, including weight control, allergy and cold medications. This method utilizes a neutralizing antibody which reduces the assay signal resulting from authentic amphetamine and methamphetamine, but not the signal resulting from cross-reactants.

This concept was implemented on Hitachi clinical chemistry analyzers using a two channel approach. Urine samples were tested using unmodified CEDIA® DAU Amphetamines reagents in one channel and reagents modified by the addition of neutralizing antibody in the second channel. The difference in rate between the two channels was calculated by the analyzer; true positive samples showed a significantly greater decrease in assay signal in response to neutralizing antibody as compared to false-positive samples.

Samples positive in an initial screen using the CEDIA® DAU Amphetamines screening test were obtained from Laboratory Specialists, Inc., New Orleans, LA. The CEDIA® neutralization method was evaluated using 158 samples that tested positive by GC/MS using the SAMHSA cutoff criteria. The neutralization test successfully identified all 158 of the GC/MS confirmed positive samples. In an analysis of 100 samples that did not confirm by GC/MS, the neutralization test successfully identified 93 of the samples as false-positives.

Samples found positive by primary screening tests for amphetamines are often re-tested with a Fluorescence Polarization Immunoassay (FPIA) amphetamine method using periodate to remove cross-reactivity from ephedrine, pseudoephedrine and phenylpropanolamine. Results from 24 samples that were positive by FPIA but negative by GC/MS showed that the CEDIA neutralization test identified 17 of these samples (71%) as false positives.

The dual channel CEDIA® Amphetamines secondary screening test can be automated on the same analyzer as is used for the initial screening method. This allows use of the same sample cup, removes chain of custody issues, improves turnaround time, and will take advantage of instrument systems that can automatically perform reflex testing.

Keywords: Amphetamine, Drug Screening, False Positive

24 Mass Spectral Stability of Drugs of Abuse Analyzed by Quadrupole and Ion Trap GC/MS Instruments

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An important criterion for acceptance of GC/MS measurements of drugs extracted from urine is stable, reproducible mass spectra. Forensic toxicology drug testing Laboratories (FTDTL) typically employ quadrupole MS systems operating in selected-ion-monitoring (SIM) mode for confirmatory purposes. An internal ionization ion trap MS, operating in selected-ion-storage (SIS) mode, was examined for its ability to produce stable ion ratios across the dynamic concentration ranges of two drug classes.

Urine specimens were prepared at five concentrations each for benzoylecgonine (BZE), amphetamine (AMP) and methamphetamine (METH). Following solid-phase extraction, the butyl derivative of BZE and the heptafluorobutyryl derivatives of AMP and METH were prepared. Hewlett-Packard 5972 and Varian Saturn 2000 GC/MS systems operating in SIM and SIS modes, respectively, were used to monitor three ions for each analyte and two for each deuterated internal standard. Concentration ranges for BZE, AMP and METH were 35-720, 50-2500 and 50-5000 ng/mL, respectively. Spectral stability was assessed by measuring extracted ion peak area ratios. Ion ratios for fifteen replicates at each concentration were compared to those of mid-range concentration calibrators. Ion ratio acceptability was defined as 20% of calibrator values.

The quadrupole MS %CV values for BZE, AMP and METH were 1-4%, 1-11% and 1-5%, respectively. There were no ion ratio failures for BZE or METH while 2 ratios failed for AMP. The ion trap MS yielded % CV values analogous to those produced by the quadrupole, 4-11% (BZE), 2-7% (AMP) and 1-6%(METH). The ion trap had 6 BZE, 6 AMP and 2 METH ion ratio failures. Overall the ion trap MS produced stable comparable mean ratios over the concentration ranges examined but had higher instability within batches. Variations in performance between drug classes were seen for both instruments. Whether or not this analyte dependency on spectral stability exists for other NIDA drugs of abuse remains to be examined.

Key Words: Ion Trap, Spectral Stability, Drug Testing

25 A Fatal Interaction between Fluvoxamine and Thioridazine

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The co-administration of thioridazine (Mellaril) and fluvoxamine (Luvox) is regarded as relatively safe due to their minimal interaction. Fluvoxamine is metabolized by the CYP 1A2 and 2D6 isozymes. Fluvoxamine is a strong inhibitor of CYP 1A2, 2C19, moderately inhibits CYP 2C9 and 3A and has mild inhibitory effects at CYP 2D6. Thioridazine is both a substrate and an inhibitor of the CYP 2D6 isozyme.

A case is presented involving a 23-year-old white male prescribed thioridazine since the age of 4 for psychoses, who was taking 400 mg/day QID prior to his death. He was also chronically prescribed valproic acid (Depakote) for the control of seizures. Eight months prior to his death he was prescribed fluvoxamine (100 mg/day) for the treatment of obsessive compulsive disorder. Within 1.5 months of his death the dose of fluvoxamine was increased to 150 mg/day. Prior to death, medical examination noted no side effects, however the family observed dry mouth, excessive drinking and tachycardia.

Death was sudden and unexpected and acute ingestion of medications was ruled out following investigation. At autopsy a slightly enlarged heart was noted, other findings were unremarkable.

Toxicological analysis of postmortem blood detected concentrations of thioridazine (7000 ng/mL) mesoridazine (1700 ng/mL), fluvoxamine (2200 ng/mL) and valproic acid (41 mcg/mL). Gastric contents were examined and found to contain less than 0.1 g of thioridazine.

The likely explanation for the high concentrations of thioridazine, mesoridazine and fluvoxamine in this case is a mild inhibition of the metabolism of thioridazine by fluvoxamine. This inhibition, although reported as mild, may slowly increase the concentrations of thioridazine and / or fluvoxamine until concentrations become toxic leading to death.

Key Words: Thioridazine, fluvoxamine, interaction

26 Detection of MDMA in Hair by LC/MS/MS

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MDMA has been a popular recreational drug in Europe for many years. The user will often report that "all is right and good with the world." It is frequently associated with all-night dance parties called "raves."

In recent years MDMA (3,4-methylenedioxymethamphetamine, "X-TC", "ecstasy", "Adam", "E") has become a common finding by forensic toxicologists as its use in the US has increased. We report a finding of MDMA in the hair of an alleged ecstasy abuser by LC/MS/MS. We believe that this is the first report of the detection of MDMA in hair that has been confirmed by LC/MS/MS.

A sample of hair was received by the laboratory for the analysis of amphetamines. Duplicate 50mg aliquots of hair were washed twice with methanol to remove any external (environmental) contamination. The hair samples were dried, pulverized, and incubated in dichloromethane. Following incubation the aliquots were then evaporated to just dryness. One aliquot was analyzed by immunoassay (EMIT). The second aliquot was derivatized with butyric anhydride and analyzed by GC/MS.

The detection of MDMA by GC/MS was confirmed by LC/MS/MS, on a third 50mg aliquot of hair. The hair sample was prepared as above. The organic solvent was evaporated to just dryness, reconstituted in mobile phase and injected on the column. The collisionally-induced dissociation transitions monitored were: propyl amphetamine (IS) 178>119, 178>91, 180>163, MDA 180>163, 180>133, MDMA 194>163, 194>133.

The LC/MS/MS procedure confirmed the presence of 110 ng/g of MDMA and a suggestion of MDA (less than 10 ng/g) in hair, suggesting the use of MDMA.

Key Words: Methylenedioxymethamphetamine (MDMA), LC/MS/MS, Hair

27 Meconium Drug Testing in Indiana

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Drug abuse during pregnancy is associated with a high incidence of asphyxia, premature birth, low fetal birth weight, congenital malformations, physical and developmental deficiencies. In order to more fully assess the prevalence of fetal growth deficiency associated with drug use during pregnancy, the State of Indiana recently established a program in which meconium specimens were required to be collected and tested for the presence of drugs of abuse from infants of low birth weight and small head circumference. The criteria for acceptance into the State Program required that the infant's birth weight be less than 2500 grams and the head circumference less than the third percentile for the infant's gestational age. The drugs/drug classes included in the testing were Amphetamines, Cannabinoids, Cocaine, Opiates, and Phencyclidine.

Screening was performed using radioimmunoassay followed by confirmatory testing by gas chromatography/mass spectroscopy. From December 1998 through June 1999 a total of 513 specimens were submitted to AIT Laboratories from within Indiana with 22.0% meeting the State Program requirements (Grant). The overall positive rate was 18.9%. Consistent with overall drug use patterns, THC was the most prevalent drug found in meconium, accounting for 61.4% of all positive specimens. This was followed by cocaine and opiates. No specimens tested thus far have been found to be positive for amphetamines or phencyclidine. These findings further support the analysis of meconium as a useful specimen for the evaluation of drug abuse during pregnancy and its incidence in infants born with low birth weight and head circumference.

	Overall	Grant	Non-Grant
Total Specimens	513	113	400
Positive Specimens*	97 (18.9%)	15 (13.3%)	82 (20.5%)
Cannabinoids	62 (61.4%)	10	51
Cocaine 36 (35.6%)	4	32	
Opiates 4 (4.0%)	1	3	
Amphetamines	0	0	0
Phencyclidine	0	0	0

* Four Non-Grant specimens were positive for both Cocaine and Cannabinoids.

Key words: Meconium, epidemiology, drug testing

28 Fatal Overdose with MDMA and GHB

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A 29 year old male was found dead in his residence following a night of partying with his friends. Postmortem specimens were submitted to our facility for toxicological analysis. Information provided by the coroner's office suggested the deceased might have used amphetamines, gamma hydroxybutyrate (GHB), and the benzodiazepine, flunitrazepam (Rohypnol ®). Initial toxicological screening using Toxi-Lab and EMIT indicated the presence of amphetamines with no other drug/drug classes detected. Blood and urine from the deceased were determined to be negative for alcohol by GC-FID, and flunitrazepam and its metabolite, 7aminoflunitrazepam by GC-ECD. Using GC/MS, the amphetamine analog, MDMA concentrations in the blood and urine were 0.88 and 86.9 ug/mL, respectively. The GHB levels in blood and urine were 1030 and 1550 ug/mL, respectively, also by GC/MS.

At autopsy, pulmonary edema and congestion were noted, as was cardiomegaly with coronary atherosclerosis and fatty liver. Furthermore, the deceased had aspirated matter in his larynx and trachea. The cause of death was determined to be the result of MDMA and GHB intoxication. The high levels of both drugs found in the urine suggest that the deceased survived for period of time after taking each of these drugs. The relative blood/urine ratios indicate that the use of MDMA may have preceded that of GHB. The current literature indicates that the MDMA and GHB levels independently in the present case may or may not have been lethal. Here we present for the first time a fatality resulting from the combined use of these drugs.

Keywords: Methylenedioxymethamphetamine,gammahydroxybuyrate, overdose

29 Analysis of Underivatized and Derivatized Sympathomimetic Amines by Capillary Gas Chromatography with Detection by Mass Spectrometry

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Sympathomimetic amine analysis by gas chromatography can be useful as both a screening and a confirmational technique. When samples are in high concentrations, as in solid dosage form, many compounds can be analyzed in their underivatized form to obtain tentative identification based on retention time and mass spectrum. Low concentration samples, such as those obtained from serum and urine extracts, are typically derivatized prior to analysis to provide better chromatography and more definitive mass spectra. Selection of an appropriate capillary gas chromatography column for this group of analytes will depend on sample concentration and specific requirements for mass spectral data.

Twenty common sympathomimetic amines, metabolites, and common interferences were chosen for this study. For high concentration samples, all compounds were analyzed as the underivatized compound on a column that had been specifically deactivated for use with basic compounds. For low concentration samples, all compounds were derivatized with either TFAA, PFPA, or HFBA. Analysis of these derivatized compounds was then performed on four separate stationary phases.

Peak shape for all of the underivatized compounds was improved by using a specially deactivated column for basic compounds. However, mass spectral information acquired during the analysis of underivatized compounds was not particularly useful in identifying unknown compounds. Better unknown identification was achieved by derivatizing this compound set. Retention time shifts and improved resolution for some compounds were noticed after analyzing all of the derivatives on each stationary phase. Some stationary phases showed unique selectivity for specific analytes.

Proper selection of a derivatizing reagent and a capillary column stationary phase can improve the resolution of specific derivatized sympathomimetic amines.

Key Words: Amphetamines, Sympathomimetics, Gas Chromatography

30 A Death due to Inhalation of Nitrogen Trifluoride

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The case presented here is about a 33 year old Caucasian male with history of depression, smoking, alcoholism, and substance abuse. He was reported missing for a couple of days by his parents. While the sheriff deputies were investigating at his home, the decedent's father found him in the backyard sitting in a chair and wearing a mask on his face. A metal tube from a tank of nitrogen trifluoride was connected to the rubber mask. Nitrogen trifluoride is used in manufacturing silicon semiconductors. The tank was empty with the valve fully open.

The autopsy showed the imprint of the mask on his face. There were a few linear superficial cuts on each wrist consistent with hesitation marks. Other than pulmonary edema and congestion, there was not any other significant finding. The decedent had talked about suicide in the past and two suicide notes were found at the scene of death.

Urine drug screen by TLC, HPLC, and immunoassays indicated the presence of acetaminophen, cannabinoids, and propoxyphene. Ethanol and other common volatiles were not found in blood. Acetaminophen and propoxyphene were not detected in blood by comprehensive basic/neutral drug screen and immunoassays. However, blood cannabinoids were positive by immunoassay. The methemoglobin concentration determined by co-oximeter was 65%. Nitrogen trifluoride is converted to fluoride in the human body. Since the body was severely decomposed; blood was unsuitable for analysis. The concentrations of

fluoride in lung and liver were 300 and 31 mg/g, respectively. The urine fluoride concentration was 13.4 mg/L or 22.3 mg/g creatinine.

The cause of death is nitrogen trifluoride poisoning and the manner of death is suicide.

Keywords: nitrogen trifluoride, fluoride, suicide

31 Oral Manifestations of Tobacco Use

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The detrimental effects of tobacco are well documented, but its use is still almost universal. Ad campaigns target young people, who are easily impressed with the perceived relationship between tobacco and pleasure, fun, well-being, sports and sexual attractiveness. Smoking is a glamorous and accepted part of society. The most reported hazards of smoking are lung and heart diseases and cancer, which are internal disorders unseen by the untrained eye. Oral damage from tobacco use in any form is visible to the untrained eye, which can help convince tobacco users of the un-glamorousness of tobacco use. Tobacco stains the teeth, produces bad breath, creates tartar problems and gum diseases, ranging from premalignant lesions to cancer. Oral cancer has a very high mortality rate, especially among latino and black ethnic groups. If not discovered in time, these diseases can lead to problems in chewing, speaking and can alter facial features. They can also involve pain, loss of time at work and can seriously affect the patient's social life. Treatment can be physically mutilating and catastrophically expensive. Oral tobacco use can even lead to death.

Keyword: Tobacco, Oral Cavity, Smoker's Mouth

32 Carbon Monoxide Stability in Stored Postmortem Blood Samples

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Carbon Monoxide (CO) poisoning remains a common cause of both suicidal and accidental deaths in the United States. As a consequence, determination of the percent carboxyhemoglobin (%COHb) level in postmortem blood is a common analysis performed in toxicology laboratories. The blood specimens analyzed are generally preserved with either EDTA or Sodium Fluoride. Potentially problematic scenarios that may arise in conjunction with CO analysis are a first analysis or a reanalysis requested months or years after the initial toxicology testing is completed; both raise the issue of the stability of carboxyhemoglobin in stored postmortem blood specimens.

A study was conducted at the Bexar County Medical Examiner's Office to evaluate the stability of CO in postmortem blood samples collected at autopsy in red top (no preservative), gray top (NaF preservative), and purple top vacutainer (EDTA preservative) tubes and submitted to the Toxicology Laboratory for analysis followed by storage at 3C. Cases for which a CO analysis was performed as part of the initial toxicological evaluation were subjected to a second CO analysis following 2 years of refrigerated storage; analyses were performed using either an IL 282 or 682 CoOximeter. The initial assay was performed on a single specimen, but all available blood specimens were assayed as part of the second CO analysis; 189 specimens from 64 cases were submitted to a second analysis.

Previous studies suggest that CO stability is affected by a number of factors. One is the volume of headspace above the specimen, which was a constant in this study because all blood specimens were stored in vacutainer tubes. A second is the presence of a preservative, although in this study the variability between first and second analyses was not related to whether specimens were unpreserved or preserved with either EDTA or Sodium Fluoride. A third is storage temperature, which was controlled in this study because all specimens were refrigerated for 2 years at 3C. A fourth is the initial %COHb, but in this study 98% of cases with initial results in the 50% - 100% range yielded second analysis results within 5% of the first result,

while this was true for only 50% of specimens from cases with initial results in the 10% - 50% range. Even greater variability occurred among specimens from cases with initial results in the <1% - 10% range. In conclusion, the results from this study suggest that carboxyhemoglobin is stable in blood specimens collected in vacutainer tubes, with or without preservative, and stored at 3C for up to 2 years, and are acceptable for either a first or repeat analysis for the determination of %COHb.

Keywords: Carbon Monoxide Stability, Carbon Monoxide Analysis, Postmortem Stability

33 The Routine Analysis of 6-Monoacetylmorphine in Urine

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The detection of 6-monoacetylmorphine (6MAM) is the primary means of detection the presence of heroin in the body. For a commercial laboratory a robust and consistent method for the detection of any drug is essential, and in these days of high sample volume and ever increasing cost pressures such methods are at a premium. The detection of 6MAM in urine requires a sensitive method with a cutoff of 10 ng/mL and reliable detection at the 4 ng/mL (40%) level. This technical note outlines the procedure to perform a routine solid phase extraction followed by the analysis of the extract on the GC/MS. Interferences are discussed and counter measures are proposed.

Solid phase extraction of 6-monoacetylmorphine (6MAM) and 6-monoacetylmorphine – D6 (6MAM-D6) utilizes GV-65 columns, from Biochemical Diagnostics, Inc., in a multiprep work station from the same supplier. Samples are buffered to pH 6.0 to optimize recovery of 6MAM. After washing with carbonate buffer (pH 9.1), methanol and hexane, the analyte is eluted with chlorobutane. 6MAM is derivatized with BSTFA and analyzed by GC-MS in Selected Ion Monitoring mode on a 15 meter HP-1 capillary column 0.25mm I.D. with 0.25 micron film. Monitored ions are 399, 340 and 400 for 6MAM and 405 and 346 for 6MAM-D6.

Hydormorphone was found to be a potential interfering substance if column condition is not optimal. To eliminate hydromorphone interference, sodium borohydride is added to the buffered samples and allowed to stand at room temperature for 30 minutes before extraction. The borohydride reduces hydromorphone to dihydromorphine, which does not interfere.

The method yields calibration curves with correlation coefficients greater than 0.98. The LOQ is 4 ng/mL, and the within-run coefficient of variation at the administrative cutoff (10 ng/mL) is 0.78%.

The 6-MAM method has been in routine use for 8 months and has performed well.

Key Words: 6-monoacetylmorphine, analysis, sodium borohydride.

34 A Case Report Involving Fluvoxamine in a Multi-Drug Associated Death

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This case report presents the toxicological findings in a poly-drug case where fluvoxamine was the major drug finding. Although the amounts of fluvoxamine were significant, the medical examiner has ruled the death accidental based upon the actions and behavior of the deceased prior to his death. The deceased was a 37 year old male who was found dead in a motel room. He had a long history of drug and alcohol use. He had previously obtained medication from doctors by faking illness. Within a week of his death, he had abused alcohol and had been treated at a crisis detox unit.

The fluvoxamine distribution was as follows: Blood (2.8 mg/L), urine (24.2 mg/L), liver (113 mg/Kg), brain (41.4 mg/Kg) and 3.0 milligrams total recovered from the stomach contents. The blood also revealed the presence of imipramine (0.12 mg/L) and diazepam (<0.1 mg/L). In addition to fluvoxamine, urine

contained imipramine (3.0 mg/L), morphine (5.1 mg/L), and codeine (0.16 mg/L). Ethanol and other volatiles were not detected.

Fluvoxamine was isolated from tissue by standard alkaline extraction and chromatographed well without derivatization. Tests were not performed to determine the metabolites. Kunsman has reported post-mortem fluvoxamine distributions in fluids such as blood, bile, vitreous fluid and urine, but the authors found no references to values in solid tissues such as brain and liver.

Key Words: Fluvoxamine, Post-mortem distribution, Fatal overdose

35 Screening of Benzodiazepines in Urine Using the OnTrak TesTstik Onsite Immunoassay

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In this presentation we report the evaluation of four lots of the OnTrak TesTstik Benzodiazepines Assay for the screening of benzodiazepines in urine. TesTstik is a simple-to-use onsite immunoassay device for the qualitative detection of single drug and/or its metabolites in urine. The cutoff value for the TesTstik evaluated was 200 ng/mL (oxazepam equivalents). Assay precision was determined by testing six urine standards that contained various concentrations of oxazepam. For the four lots evaluated, 100% of the tests were negative with drug at 50 ng/mL, 80-100% were negative with drug at 100 ng/mL, and 98-100% of were positive with drug at 300 ng/mL. The clinical performance was assessed using 50 GC/MS-confirmed positive clinical samples and 100 samples that were screened negative for benzodiazepines in a clinical laboratory. There was a 100% correlation between TesTstik and classic Abuscreen OnTrak benzodiazepines assays. One lot of TesTstik was used to screen 500 urine samples that had been screened negative for the NIDA-5 drugs in a clinical laboratory. Fifty of the 500 samples produced positive results by TesTstik and were further tested using Abuscreen OnLine benzodiazepines assay with glucuronidase treatment. Forty one of the 50 samples tested positive by OnLine and the remaining 9 samples exhibited benzodiazepines values ranging from 57 to 94 ng/mL. Assay specificity was determined using panels of structurally related and non-related compounds and "Approximate Percent Cross-reactivity" was calculated from the concentration that produced a positive result (equivalent to 200 ng/mL oxazepam). The average value of approximate cross-reactivity for each of the structurally related compounds is listed below: -Hydroxyalprazolam, 140%; 4-Hydroxyalprazolam, 125%; Alprazolam, 65%, Bromazepam, 175%; Chlodiazepoxide, 15%; Clorazepate, 60%; Demoxepam, 30%; 7-Amino-1-Desmethylflunitrazepam, 130%; 7-Aminoflunitrazepam, 200%; Desmethylflunitrazepam, 75%; 3-Hydroxy-flunitrazepam, 25%; Flurazepam, 85%; 7-Acetamidoflunitrazepam, 0.2%; Hydroxyethylflurazepam, 200%; Didesethylflurazepam, 165%; Norfludiazepam, 90%; Lorazepam, 95%; 7-Aminonitrazepam, 125%; 7-Acetamidonitrazepam, 0.4%; Nordiazepam, 90%; Alpha-Hydroxytriazolam, 105%; Temazepam, 80%; 4-Hydroxytriazolam, 65%; Triazolam, 45%. In conclusion, the TesTstik benzodiazepines Assay can serve as a useful tool for the rapid, onsite screening of benzodiazepines in urine.

Key Words: Benzodiazepines; Onsite Immunoassay; TesTstik.

36 Simultaneous Detection and Quantitation of Diethylene Glycol, Ethylene Glycol and the Toxic Alcohols in Serum using Capillary-Column Gas Chromatography.

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Determination of toxic glycols and alcohols in an emergency setting requires a rapid, yet accurate and reliable method. We modified the gas-chromatographic (GC) method of Livesey et al (Clin Chem 1995;41:300-305) to simultaneously determine Diethylene Glycol (DEG) along with ethanol, methanol,

isopropanol and acetone, and ethylene glycol. The system uses a Hewlett Packard (HP) 6890 GC with EPC, a Gooseneck Splitless Liner (Restek), and a Rtx-200 capillary (Restek) column (30 m x 0.53 mm i.d., 3 m). After serum samples are deproteinized using ultrafiltration (Millipore ULTRAFREE-MC), 1 L of the protein-free filtrate is manually injected. Internal standards for alcohols (and acetone) and glycols are n-propanol and 1,3-butanediol, respectively. All compounds elute within 3.5 minutes (linear temperature gradient from 40 to 260 ^{o}C); total run time is 6.5 minutes. Limit of detection and linear range for all compounds are 5 mg/dL and 0-500 mg/dL, respectively. Retention times and day-to-day precision (CV) using two levels of a commercial control (n=10) are given below.

Compound	Retention time(min)	UTAK Volatiles Plus 10 mg/dL - CV (%)	UTAK Volatiles Plus 25 mg/dL - CV (%)
Methanol	0.84	3.7	7.3
Ethanol	1.07	3.4	8.6
Isopropanol	1.28	5.3	7.5
Acetone	1.76	4.8	9.6
Ethylene Glycol	2.59	9.8	4.0
Diethylene Glycol	3.44	9.8	8.8

In addition there is no interference from propylene glycol and 2,3 butanediol (other compounds of clinical interest). We conclude that use of the HP 6890 GC (splitless mode) with modification in the temperature program allows increased resolution and thus, detection and reliable quantitation of DEG and other toxic glycols and alcohols of clinical interest. The method is in clinical use for patients seen in our emergency department.

Keywords: alcohols, glycols, gas chromatography

37 Toxicologist found dead in Puerto Rico, Scuba Accident Under Investigation

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This presentation is the third annual murder mystery poster. Should you decide to participate, you will get a packet of information that includes the case background and a few clues. The rest of the clues are found at various vendor booths located at the conference. Your task, should you decide to accept it, will be to collect the remaining clues that will assist you in solving the problem and return them with your answer to the postersite.

Key Words: murder, mystery, clues

38 Methamphetamine Disposition in Saliva and Plasma.

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Methamphetamine has been detected in saliva collected from drug abusers. However, limited research has been performed to characterize the pharmacokinetics of methamphetamine and metabolite secretion in saliva following controlled drug administration to human volunteers. This report describes the disposition of methamphetamine and its primary metabolite, amphetamine, in plasma and saliva following oral methamphetamine administration. Volunteers received low dose (10 mg) and high dose (20 mg) of (d)-methamphetamine HCl formulated in Desoxyn Gradumet tablets. Blood and stimulated saliva specimens were collected simultaneously and analyzed by solid phase extraction followed by GC-MS. The lower limit of quantitation for both analytes was 2.5 ng/mL. Standard curves were linear across a concentration range of 2.5-500 ng/mL with correlation coefficients = 0.985. At the present time, two participants have completed the 10 mg dose and one subject has completed the 20 mg dose.

Methamphetamine was first detected in plasma between 1 to 2 h (range 6.4 to 14.2 ng/mL) while amphetamine was not detected until 4 to 12 h at lower concentrations of 2.6 to 6.2 ng/mL. Peak plasma methamphetamine and amphetamine concentrations occurred at 4 to 8 h (range 15.7 to 36.1 ng/mL) and 4 to 12 h (range 2.6 to 6.2 ng/mL), respectively. Methamphetamine was detected in plasma throughout the 24 h sampling period, while amphetamine was detected for up to 12 to 24 h. Methamphetamine was first detected in saliva between 0.5 to 1 h (range 5.1 to 147.5 ng/mL). Amphetamine was not always detected after oral methamphetamine administration. Peak saliva methamphetamine and amphetamine concentrations occurred at 2 to 8 h (range 90.2 to 315.5 ng/mL) and 4 to 8 h (range 15.0 to 21.3 ng/mL), respectively. Methamphetamine and amphetamine were detected in saliva for up to 24 h. Saliva/plasma methamphetamine ratios were generally greater than 1, although ratios varied considerably over time.

In addition, inter-subject variability was greater for saliva methamphetamine than plasma methamphetamine concentrations. Parent methamphetamine concentrations were always higher than amphetamine plasma and saliva concentrations throughout the 24 h measurement period. Although these data are limited, we have begun to characterize the disposition of methamphetamine and amphetamine in plasma and saliva following oral drug administration.

Keywords: Methamphetamine, Saliva, Plasma

39 Isolation and Identification of 4-Hydroxyclobenzorex Following Administration of Clobenzorex

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Clobenzorex (Asenlix) is an anorectic drug that is metabolized to amphetamine which causes difficulty in interpretation of amphetamine positive drug tests. Previous studies have shown the parent drug and several metabolites are excreted in urine. Clobenzorex has been reported to be detected for only a short time (up to 4 h post-dose), however, the short detection time may have been due to the fact that 4 hour rather than individual samples were collected and the detection limit was 50 ng/mL. In a more recent study, clobenzorex was detected for as long as 29 h post-dose owing to a 50 fold increase in sensitivity (to 1 ng/mL). Despite the resultant increased detection times, several amphetamine positive samples (500 ng/mL) contained no detectable clobenzorex. Thus, the absence of clobenzorex in the urine does not exclude the possibility of its use.

To more definitively establish the use of clobenzorex (or lack thereof), evaluation of another metabolite was considered. An hydroxy metabolite has been reported in the urine of some subjects as long as amphetamine. However, quantitative analyses were not performed in this report and the position of the hydroxyl group was not established. 4-OH-clobenzorex, synthesized for this study, proved to be easily detected and was typically found at levels higher than amphetamine, long after clobenzorex itself was no longer detected, making it a better indicator of clobenzorex use.

Samples obtained from a controlled study involving the administration of clobenzorex (30 mg) were analyzed for the presence of the hydroxy metabolite. Since hydroxy metabolites are excreted as conjugates, acid or enzyme hydrolysis was performed. Dramatic differences were observed depending on the hydrolysis method. The amount of free hydroxyclobenzorex after acid hydrolysis increased dramatically at higher temperatures with autoclaving the most effective. Enzyme hydrolysis was also effective but commonly, the two methods gave substantially different results. Unfortunately, neither method consistently gave the greatest degree of hydrolysis for all samples analyzed. The procedure for detection of 4-OH-clobenzorex consists of liquid-liquid extraction and GC/MS by monitoring ions at m/z 118, 125, 330 and 364. 4-OH-clobenzorex and its 3-Cl regioisomer were used in the identification and quantitation of the metabolite.

Key Words: Hydroxyclobenzorex, Clobenzorex, Amphetamine

40 Elimination of Cocaine and Metabolites in Plasma, Saliva and Urine Following Repeated Oral Administration

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Chronic administration of lipophilic drugs can result in accumulation of the drug in the body and prolonged elimination upon cessation of use. For cocaine, it has been suggested that cocaine and/or metabolites can be detected in saliva and urine for an extended period following long-term, high-dose administration. We investigated the effects of chronic oral cocaine administration in healthy volunteer subjects with a history of cocaine abuse. Subjects were housed on a closed, clinical ward and were administered oral cocaine in up to 16 daily sessions. In each session, volunteers received 5 equal doses of oral cocaine with 1 hour between doses. Across sessions, cocaine was administered in ascending doses with an initial dose of 100 mg (500 mg/day) up to 400 mg (2g/day), increasing by 25 mg/dose/session (125 mg/session). Participation in the study was terminated if cardiovascular safety parameters were exceeded. Plasma and saliva specimens were collected at designated periods during the dosing sessions and during the one week withdrawal phase at the end of the study. All urine specimens were collected throughout the entire study. Specimens were analyzed for cocaine and metabolites by solid phase extraction followed by GC-MS analysis in the SIM mode. Limits of detection for each analyte were approximately 1 ng/mL. The analytes detected included benzoylecgonine (BZE), ecgonine methyl ester (EME), cocaine (COC), benzoylnorecgonine, norcocaine, *m*- and *p*-hydroxycocaine and *m*- and *p*-hydroxybenzoylecgonine.

Noncompartmental analysis was employed for the determination of plasma and saliva pharmacokinetic parameters. Urinary elimination half-lives for cocaine and metabolites were determined by constructing ARE (amount remaining to be excreted) plots. An initial elimination phase was observed during withdrawal that was similar to that observed after acute dosing. The mean (N=6) plasma, saliva and urine cocaine elimination half-lives during the initial phase were 1.5±0.1 h, 1.2±0.2 h and 4.1±0.9 h, respectively. A biphasic urinary elimination of cocaine and metabolites was also observed. The mean (N=3) urinary cocaine elimination half-life for the secondary phase was 19.0±4.2 h. There was some difficulty in determining if a secondary elimination phase was present for the remaining three subjects because of interference by high concentrations of BZE. A secondary elimination phase was also noted for most metabolites with half-life estimates ranging from 14.6-52.4 h. These secondary elimination half-lives greatly exceeded previous estimates from studies of acute cocaine administration. These data suggest that cocaine accumulates in the body with chronic use, resulting in a prolonged terminal elimination phase for cocaine and metabolites.

Key words: Oral Cocaine, Chronic Cocaine, Plasma, Saliva, Urine

41 Sweat Patches: Association Between Length of Wear and Concentrations of Cocaine, Benzoylecgonine, and Ecgonine Methylester.

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Sweat patches are an innovative method of assessing drug use. An important scientific and practical question is the minimum length of time a patch needs to be worn under actual field conditions to detect cocaine use. 22 active drug users and 5 non-using comparison subjects wore short-term patches (½ hr., 1hr., 1½ hr. and 2 hr.), followed by long-term patches (1, 3, 7, 14 day patches). Short- and long-term patches were identical except for intended length of wear. Data were collected at a storefront in East Harlem, New York City (a high dug use neighborhood.) Outreach workers, known in the community and in contact with various types of drug users obtained subjects in four approximate categories: 1) irregular (monthly or weekend) cocaine users, 2) near-daily cocaine snorters, 3) speedball users (inject cocaine with heroin), and 4)

crack smokers . Categories were imprecise because it was dependent upon self-report at point of acceptance into the study.

No detectable cocaine analytes were found in 107 short-term patches. No detectable benzoylecgonine (BE), or ecgonine methylester (EME), and only trace levels of cocaine (COC) were found in the 98 long-term patches worn by the comparison group while higher concentrations of all three analytes were found in 408 long-term patches of drug users by GC/MS. Further analyses were conducted on these 408 long-term patches. Two types of hierarchical regression analyses were performed on the log of the concentrations of each analyte. First, for all three analytes, the significant differences for type of patch were: 1-day > 0, 3-day > 1-day, 1 wk. > 3-day. (However, only for BE was 2-week > 1-week.) Average concentrations of COC, BE and EME were: 1-day 325, 59, 30 ng/mL; 3-day 817, 168, 83 ng/mL; 1 wk. 1196, 182, 117 ng/mL; 2-wk. 1664, 724, 211 ng/mL. Second, type of patch was replaced as an independent variable by number of minutes of wear time. Although observations suggested the possibly of curvilinearity, only the linear trend was significant. On two randomly selected days, pairs of 1-day patches were applied concurrently to assess the reliability of 1-day patches. Correlations between patch Ln(analyte) for each day were: COC, .90, .91; EME .78, .82, : and BE, .86, .93.

Conclusions: The minimum length of wear required for detection of cocaine use in this sample was greater than 2 hrs. and less than or equal to 1 day. Between 1 day and 2 weeks, Ln(analyte concentrations) increases as a linear function. 1-day patches have acceptable reliability. Sweat patches are an important, new method of assessing drug use.

KEYWORDS: Sweat patch, drug use detection, cocaine

42 Sweat Testing for Cocaine and Metabolites with Hand-held and Torso Fast Patches

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Sweat testing for drugs of abuse provides a convenient and less invasive method for monitoring drug exposure than blood or urine. The most common collection device is the PharmChek Sweat Patch usually worn for 7 days. Two new devices, the Hand-held Fast Patch and the Torso Fast Patch, employ heat-induced sweat stimulation and a larger cellulose pad to increase sweat collection and reduce collection time to 30 min. Subjects (N=4) with histories of cocaine and opiate use provided informed consent and were paid for their participation in this 10-week clinical research study. Sweat specimens were collected up to 29 days after 3 low (75-mg/70 kg) and 3 high (150-mg/70 kg) cocaine HCL doses administered by the subcutaneous route. Cocaine and metabolites were determined simultaneously by GC-MS following solid-phase extraction. The primary analyte detected in sweat was cocaine with peak concentrations ranging from 33-3579 ng/patch across doses for the Hand-held Patch compared to 22-2085 ng/patch for the Torso Fast Patch. Peak concentrations generally occurred within 24 h after dosing but were highly variable within and between subjects without a clear dose-response relationship. In comparison, cocaine was detected from 1 to 20 days after the low dose and 1.7 to 10 days after the high dose in Torso Fast Patches.

Cocaine was detected in Hand-held Patches throughout the measurement period of 24 days after the low and 17 days after the high dose in 3 of 4 subjects. Considerably lower concentrations of metabolites were also present in some patches. Generally, concentrations of cocaine were higher in Hand-held than Torso Fast Patches and considerably higher than those reported in PharmChek Sweat Patches (peak cocaine concentrations 10-45 ng/patch after 25 mg IV cocaine hydrochloride, Cone, et. al, JAT 18:298, 1994). These interesting preliminary findings indicate that stimulation with heat substantially increases the amount of drug secreted, reduces the time necessary to collect sweat and greatly extends the detection time following drug usage.

Keywords: Cocaine, Sweat, Hand-held Fast Patch, Torso Fast Patch

43 Sweat Testing for Cocaine with the PharmChek™ Sweat Patch.

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This report describes cocaine and metabolite disposition in sweat collected from 4 human subjects during a 10-week inpatient study. All subjects had a recent history of cocaine use and tested positive for cocaine metabolite by urinalysis prior to beginning the study. The first 3 weeks served as a washout phase followed by administration of 3 doses of cocaine HCl (75 mg/70 kg, subcutaneous) on alternating days in week 4 (low dose week). The same dosing sequence was repeated in week 8 with doubled doses (high dose week). Sweat was collected with PharmChek patches worn on the side of the abdomen for 1-week intervals; a new patch was applied once each week. Patches were extracted with acetate buffer (pH 4.0, 0.5 M) followed by solid phase extraction and GC-MS analysis for cocaine and metabolites. Cocaine was detected in patches worn by only 1 subject during week 1 (78.4 ng/patch) and week 2 (7.4 ng/patch) of the washout phase. Patches worn by all subjects in week 3 tested negative. Cocaine concentrations in patches worn during week 4 (low dose week) ranged from 0-89 ng/patch. In week 5, patches were applied 4 days after the last low dose and worn for the next 8 days. Drug concentrations in these patches were near or below the assay LOD (2 ng/patch). All patches worn during weeks 6 and 7 tested negative. Cocaine concentrations in patches worn during week 8 (high dose week) ranged from 11-298 ng/patch. Only 1 patch worn by a subject in week 9 (from 4-12 days after the last high dose) contained cocaine (19.7 ng/patch). All patches worn during week 10 tested negative. Benzoylecgonine and ecgonine methyl ester were the primary metabolites in patches; metabolite concentrations ranged from 0-81% of cocaine concentrations. Overall, there was considerable inter-subject variability in drug accumulation in patches after dosing. These preliminary data suggest that sweat testing with the PharmChek patch is primarily effective in detecting cocaine use that occurs while the patch is worn.

Key Words: Sweat, Cocaine, Patch

44 Sweat Testing for Methamphetamine with the Hand-held Fast Patch

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An inpatient, clinical study is in progress to characterize the disposition of methamphetamine, cocaine, and codeine in biological fluids and tissues. This report describes secretion of methamphetamine and its major metabolite, amphetamine, in sweat after oral administration of low (10 mg) and high doses (20 mg) of (d)-methamphetamine HCl formulated in Desoxyn Gradumet tablets. One subject received low and high doses; one subject received only the low dose. Sweat was collected from the palm with a heated sweat patch device (Hand-held Fast Patch) for 30-min intervals prior to and following drug administration. Patches were extracted in acetate buffer (pH 4.0, 0.5 M) followed by solid phase extraction. Extracts were derivatized by a dual derivatization method (MTBSTFA and BSTFA) and analyzed by GC-MS for methamphetamine and amphetamine. Methamphetamine was initially detected in specimens collected within 2-4 hr after the low dose (12-26 ng/patch) and high dose (64 ng/patch). Amphetamine was initially detected in specimens collected within 4 hr following both doses, and concentrations in these specimens were typically near the assay LOD (ca. 1 ng/patch). Methamphetamine and amphetamine secretion in sweat peaked within 4-21 hr after drug administration. Peak methamphetamine concentrations ranged from 52-78 ng/patch following the low dose; the peak concentration following the high dose was 320 ng/patch. In comparison, peak amphetamine concentrations ranged from 0-6 ng/patch following the low dose with a peak concentration following the high dose of 6 ng/patch (< 2% of methamphetamine concentrations). After methamphetamine concentration peaked, there was an initial rapid decrease in concentration over the first 24 hours. However, this was followed by a more gradual decline with drug detected for at least 3 days post-drug administration. These preliminary data indicate that sweat testing for methamphetamine with the Hand-held Fast Patch may be a practical alternative to drug testing with other matrices. The Hand-held Fast Patch is a rapid,

noninvasive specimen collection device, and methamphetamine can be detected in sweat collected with the Hand-held Fast Patch for at least 3 days after methamphetamine use.

Keywords: Sweat, Methamphetamine, Hand-held Fast Patch

45 Deterrent Effects on Drug Positive Rates Using the PharmChek Sweat Patch

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The PharmChek Sweat Patch is a non-occlusive Band-Aid type of device used to collect both sensible and insensible sweat. The properties of the sweat patch allow the device to be worn for extended time periods (up to several weeks) and contains tamper-evident properties, which can indicate if the patch has been removed or adulterated. The sweat patch is capable of detecting drug use 1-3 days prior to the sweat patch application and up to 24 hours prior to the sweat patch removal. The sweat patch was cleared by the FDA as a collection device in 1990 and in 1995/1996 for the detection of amphetamine, methamphetamine, cocaine, benzoylecgonine, codeine, morphine, heroin, 6-AM, THC and phencyclidine. The sweat patch has been used in Family Courts and Child Protective services since 1997 to monitor parents/guardians who have been involved with the use of illicit drugs. These individuals are monitored for drug use using the sweat patch to demonstrate that they are drug free and to subsequently regain parental/guardian privileges. As noted in a previous study conducted by the Michigan Department of Corrections, the sweat patch has a significant deterrent effect on drug positive rates. In this study which ran between 3 and 6 months, positive drug rates decreased by 53 % while the patch was worn. In the current study involving Family Courts and Child Protective Services, which covers an 18-month time period, the overall patch positive rates went from 39.5% to 17.4%. Positive drug rates by drug/month/quarter are presented. The decrease in drug positive rates has been primarily attributed to the deterrent aspects of the sweat patch as they relate to behavior modifications involving illicit drug use.

Key words: Sweat Patch; Deterrent; Positive Rates

46 Cocaine and Metabolite Elimination Patterns in Heavy Cocaine Users during Cessation I: Saliva and Plasma Analysis

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Earlier reports have suggested that cocaine may accumulate in tissues following chronic administration and be detectable for an extended period of time compared to acute dosing. A study was designed to investigate the elimination patterns of cocaine and metabolites in the body fluids of chronic high-dose cocaine users. Male and female cocaine-using volunteers (N=20; 13 men) were housed on a closed research ward where blood and saliva specimens were collected periodically for up to 12 hours, in the absence of drug administration, beginning immediately upon their entry to the ward. Blood specimens were treated with sodium fluoride and acetic acid. Saliva specimens were untreated. All specimens were frozen and stored for later GC-MS assay (LOQ for all major metabolites 1.25 ng/mL). Subjects had mean ± SD histories of 7.8-± 4.9 years duration of cocaine use. Mean current cocaine use was 9.9 ± 3.1 days in the past 2 weeks and 1.1 ± 0.7 grams per day (range of 0.12 to 2.5 grams). The mean number of drugs (other than cocaine and alcohol) used was 1.3; 13 subjects had histories of opioid use with 4 subjects reporting recent use. Time since last reported use of cocaine prior to sample collection was 16.77 ± 15.32 hours with a range of 2.5 to 63 hours.

Plasma cocaine (COC) was detected in 16 subjects with an initial mean of 13.4 ± 37.0 ng/mL (highest-162.2). Four subjects were negative for all analytes upon admission. Mean (highest) intake

concentrations (ng/mL) of the main analytes detected in plasma were: benzoylecgonine (BE) 317.3 ± 353.4 (1292.3); and ecgonine methyl ester (EME) 32.4 ± 66.8 (289.75). Mean (highest) values in saliva were: COC 19.5 ± 41.4 (170.7); BE 47.4 ± 46.0 (144.4); and EME 34.6 ± 84.4 (337.0). Other metabolites detected in plasma-included m- and p-hydroxycocaine and m- and p-hydroxybenzoylecgonine, norcocaine, and benzoylnorecgonine. Where cocaine was detected in both matrices, mean and range of intake saliva-to-plasma (s/p) ratios were: COC 5.2 ± 6.1 (0.7 to 16.1); BE 0.1 ± 0.2 (0 to 0.6); EME 0.6 ± 0.4 (0 to 1.4). Cocaine was detected in the saliva and blood of 6 of the 20 subjects at 12 hours after entry. These naturalistic data suggest those low concentrations of unmetabolized cocaine and higher concentrations of metabolites can be detected in both plasma and saliva several hours after last use. While cocaine s/p ratios were comparable to those reported from studies of acute administration, these data suggest that EME accumulate as a result of chronic administration.

Key words: cocaine, elimination, plasma, saliva

47 Quantitation of Cocaine and Three Metabolites in Blood, Urine, and Milk, Using GC-MS

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A simple and widely used solid-phase extraction procedure (United Chemical Technologies Method Handbook) was applied for the GC-MS identification and quantitation of cocaine (COC), benzoylecgonine (BE), cocaethylene (COCE), and m-hydroxybenzoylecgonine (HBE) in blood, urine, and milk. The method utilizes BSTFA as a derivatizing agent, which yielded abundant diagnostic ions with high *m/z* values.

Linear quantitative response curves were generated for the compounds of interest over a concentration range of 5-200 ng/mL. Linear regression analyses of the standard curve in the three specimen types exhibited correlation coefficients ranging from 0.996 to 1.000. The LOD values for COC, COCE, and derivatives of BE, and HBE in the three specimen types ranged from 0.9 to 21 ng/mL. The LOQ values, however, ranged from 1.1 to 29 ng/mL. Accuracy (within-run and between-run) studies reflected a high level of reliability and reproducibility of the method. Within-run accuracy ranged from 91.1 to 93.2 %, and between-run accuracy ranged between 87.7 and 93.3%.

The applicability of the method for the detection and quantitation of COC, BE, COCE, and HBE was demonstrated successfully for postmortem human blood and urine samples, as well as *in vivo* blood samples obtained from rats subcutaneously administered cocaine (40 mg/kg). To the best of our knowledge, this the first time that the presence of HBE in postmortem blood specimens has been investigated. Supported by NIH/NCRR 1P20RR11104-04 grant and MSM Faculty Development grant.

Key Words: Cocaine, Solid-Phase Extraction, and Cocaine Metabolites.

48 Development of a Nitrite-Detect Test for the Automated Chemistry Analyzers to Test Urine Samples Adulterated with Nitrite Based Adulterants.

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Specimen tampering is a growing problem for the drug testing laboratories. Implementation of pre-employment drug testing by many industries contributed to an increase in adulteration of samples to escape drug detection. Adulteration of samples can be simple dilution, substitution or addition of a chemical agent. Currently, the most prevalent chemical agent added to urine is the product called Klear. The adulterant Klear can be purchased via Internet and is supplied as a package of two vials, each containing 500 mg white powder. The white powder in Klear has previously been identified as Potassium Nitrite. Whizzies is another nitrite-based adulterant that contains Sodium Nitrite. Users of Klear are instructed to add the contents of one vial to 4 ounces of urine. The addition of nitrite does not cause a change in color, pH or

specific gravity of the urine but it interferes with the detection of most drugs with THC being the most affected. The samples that are detected as positives by immunoassay fail to be confirmed by GC/MS because of the destruction of the internal standard by the nitrite in the sample.

We have developed a method for the automated chemistry analyzers to test for nitrite levels in urine samples adulterated with nitrite based adulterants. The method is based on the Griess reaction in which the nitrite in the sample forms a diazonium compound that produces pink color by complexing with the indicator dye N-(1-napthyl)ethylenediamine. The absorbance at 540nm is directly proportional to the nitrite concentration in urine. The method employs one reagent system and consists of a ready-to-use liquid reagent and calibrators. Both the within-run and between-run precision (%CV) for low and high controls is < 5%. No significant interference was observed with the endogenous substances. The method is linear over the concentration range 16-1000ug/mL with a correlation coefficient of 0.999. The limit of detection of the test is 6 ug/mL. The accuracy of the method was determined by testing the recovery of spiked samples and correlation of 110 samples with a commercially available method. Recovery of the spiked samples at 150-2500ug/mL ranged from 94-107% and a sample correlation of 0.999 was obtained.

In conclusion, the Nitrite-Detect Test is applicable to several automated chemistry analyzers. It is a convenient, reliable, precise and economical method to detect nitrite in urine samples adulterated with Klear.

Key Words: Klear, Nitrite, N-(1-napthyl)ethylenediamine.

49 Laboratory Experience with Adulterated Blind Quality Control Samples

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One of the major challenges confronting employers and drug testing laboratories over the past few years is the widespread proliferation of the availability of adulterants and their use by persons being tested. It is estimated that nearly 1,000 adulterated specimens are submitted to drug testing laboratories each week.

On September 28, 1998, the Department of Health and Human Services (HHS) issued a guidance document to HHS-certified laboratories for reporting specimen validity test results and, in a parallel document for MROs, the Department of Transportation (DOT) issued guidance for interpreting specimen validity test results. The HHS and DOT regulations permit laboratories to test specimens for dilution and adulteration, but do not require federal agencies or transportation employers to order such tests. Many employers have asked their contract laboratories to do validity testing and many laboratories offer such tests.

In order to evaluate the status of employer concern about adulteration and the ability of laboratories to detect drugs in the presence of adulterants and adulteration, four blind quality control samples were prepared, each containing THC-9-carboxylic acid (ca. 50 ng/mL) and hexahydrocannabinol-9-carboxylic acid (ca. 200 ng/mL), to which was added the following adulterants: nitrite, acid, alkali, and detergent (clear dish soap). The samples were bottled in 60-mL volumes and frozen until prior to submission to laboratories.

Starting in October 1998, 3 to 5 samples of each type were prepared as blind QC samples and submitted each month to several of 18 different HHS-certified laboratories through a number of different companies. MROs sent the results to Duo Research for decoding and evaluation. Whenever possible, laboratory inspections were conducted to determine how the samples were tested and to evaluate any erroneous results.

The sample containing nitrite was never reported as positive for cannabinoids, apparently due to significant oxidation of the cannabinoids in the 24-hour transit period to the laboratories. Some laboratories identified the presence of nitrite. The acid and alkali samples gave the most cannabinoid positive results, with some laboratories reporting the adulterations. The sample containing detergent gave laboratories the most problems, with the sample usually screening positive, but not giving a successful confirmation. Only one laboratory reported the presence of soap. Also, many employers have not requested adulteration testing.

Data comparing laboratory performance and testing issues will be presented.

Key Words: Drug Testing, Cannabinoids, Adulteration

50 Testing of Post-mortem Samples with the EMIT® II Plus Ethyl Alcohol Assay

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The EMIT II Plus Ethyl Alcohol Assay consists of liquid, ready-to-use reagents and calibrators with excellent storage and calibration stability. The reagents have shown less than 10% rate loss during nine months of storage, which should result in a minimum of 18 months refrigerated stability. On the SYVA-30R, these reagents give greater than 50 days calibration stability. This assay represents a significant improvement over the EMIT Ethyl Alcohol Assay in that post-mortem blood samples do not produce false positive results. The falsely positive values were a result of the presence of lactate dehydrogenase (LDH) enzyme and lactate in post-mortem samples. The EMIT II Plus Ethyl Alcohol assay effectively eliminates LDH activity from patient samples. Nine alcohol-free post-mortem blood samples were diluted 1:3 with negative calibrator or water and tested with both assays on the SYVA-30R analyzer. These samples, which quantitated between 190 and 435 mg/dl using the EMIT reagents, all gave reported ethanol results of zero or less in the new EMIT II Plus assay. In addition, the EMIT II Plus reagents demonstrated recovery between 80-120% when these samples were spiked to 100 mg/dl ethyl alcohol, diluted as above, and tested on the SYVA 30-R, Hitachi* 717, and Hitachi 911 analyzers. Nineteen post-mortem samples containing 60-340 mg/dL ethanol (by headspace GC) gave average recoveries of 80-88%, with correlation coefficients of >0.93, when tested with the EMIT II Plus assay on these three analyzers. Within-run C. V.'s of <7% for postmortem samples containing approximately 80 and 170 mg/dL ethanol were obtained with the EMIT II Plus Ethyl alcohol assay on all three analyzers.

* Is a trademark of Roche Diagnostics Boehringer Mannheim Corporation

Keywords: Ethyl Alcohol, Post-mortem samples, EMIT II Plus Assay

51 A Comparison of Roche Kinetic Interaction of Microparticles in Solution (KIMS®) Assay for Cannabinoids and GC/MS Analysis for 11-nor-9-carboxy- delta 9-tetrahydrocannabinol

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In this study, we investigated the effectiveness of the Roche Kinetic Interaction of Microparticles in Solution (KIMS®, Roche Diagnostics, and Montclair, NJ) screening assay for cannabinoid metabolites (THC). Urine specimens were collected from subjects participating in a controlled clinical study of smoked marijuana conducted on the closed research ward of the Intramural Research Program, NIDA. Subjects provided informed consent and were paid for their participation. All urine specimens (N = 1427) were collected from 25 frequent marijuana smokers. These specimens were collected during the elimination time period of THC smoked prior to entry onto the clinical ward and following controlled administration of a single marijuana cigarette. Specimens were analyzed concurrently for THC by a customized Department of Defense (DOD) cannabinoid KIMS® kit (50 ng/mL cutoff) formulated to increase THC sensitivity and for 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH) by GC/MS (15 ng/mL cutoff) at the US Army Drug Testing Laboratory at Tripler Army Medical Center. Sensitivity of the KIMS assay was 69.7% as calculated by (TP/(TP + FN))*100 where TP (true positive) = 389, FN (false negative) = 169 and specificity was 99.8% as calculated by (TN/TN + FP)*100 where TN (true negative) = 867 and FP (false positive) = 2. Many of the false negative results had GC/MS concentrations between 15 and 25 ng/mL (N=151). These results suggest that the current DOD screening test for THC may fail to adequately identify potential positive specimens in the 15-25 ng/mL THCCOOH concentration range. A further evaluation of the KIMS® THC screening assay is warranted.

Keywords: Cannabinoid, Marijuana, Tetrahydrocannabinol, Immunoassay,

52 Determination of Methadone Metabolite(EDDP) with DRI Enzyme Immunoassay

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Methadone, a synthetic opiate has been used in the treatment of heroin addiction. Screening for Methadone or its metabolite is essential in determining patient compliance to treatment. Various immunoassay techniques are available for Methadone compliance monitoring. However these tests measure the parent drug only (i.e. Methadone) and are subject to "false positive" from addicts who add a portion of their methadone directly into the urine sample. As a result, confirmation of the presence of EDDP by Thin Layer Chromatography (TLC) or Gas Chromatography (GC) is often required. Determination of the presence of EDDP in urine with an immunoassay will make possible the widespread testing for compliance and can rule out the possibility of urine adulteration in clinics where unsupervised urine collections are permitted.
DRI has developed a homogeneous enzyme immunoassay for the detection of Methadone Metabolite in urine with minimum cross reactivity towards the parent drug (i.e. Methadone). The liquid ready-to-use reagent requires no preparation and is applicable on various clinical chemistry analyzers. The assay performance and correlation with GC are summarized below.

Sensitivity: 75ng/ml

Precision: within-run; n=20

Calibrator (ng/ml)	Means.d. (%cv)
0	6204.1(0.7%)
300	7764.4(0.6%)
1000	9747.7(0.8%)
2000	10835.4(0.5%)

Accuracy and Correlation:

		GC	
Methadone Metabolite EIA		Positive	Negative
DRI	Positive	55	2*
	Negative	0	40
	% Agree	98%	

*DRI EIA results were 1000ng/mL, GC values were 800ng/ml and 900ng/ml respectively.

We conclude that DRI Methadone Metabolite assay is a convenient, rapid, and accurate method for urine Methadone Metabolite screening.

Key Words: Methadone Metabolite (EDDP), Gas Chromatography (GC), andThin Layer Chromatography (TLC)

53 CEDIA® 6-Acetyl Morphine Assay for Urine Drug Testing

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We have developed a homogeneous enzyme immunoassay for the heroin metabolite 6-acetyl morphine (6AM) based on the CEDIA® technology. Unlike currently available assays for opiates, which detect morphine, codeine and a wide variety of other opiates and their metabolites, the CEDIA® 6AM assay allows specific identification of heroin abusers. This eliminates confusion with patients taking prescription pain medication (e.g., codeine, and hydrocodone), and with individuals claiming to test positive due to ingestion of foods containing poppy seeds.

In the CEDIA® 6AM assay, the enzyme ß-galactosidase is split into two inactive fragments: a large fragments (EA) and a smaller polypeptide (ED), which can spontaneously recombine to form active enzyme. The 6AM analyte is covalently attached to each ED molecule so that enzyme formation is not affected;

however, binding of the ED-6AM conjugate by a highly specific monoclonal antibody to 6AM inhibits re-association of enzyme fragments. Analyte present in a calibrator or sample competes with the ED-6AM conjugate for binding to the antibody. Thus the amount of enzyme formed (as measured by the rate of substrate hydrolysis) is proportional to the analyte concentration. The CEDIA® 6AM assay is a homogeneous method that can be performed on a wide variety of clinical chemistry analyzers.

Studies to date have been performed on the Hitachi 747 and Hitachi 717 analyzers. The CEDIA® 6AM assay uses a 10 ng/mL cutoff calibrator and has a range of 0 to 30 ng/mL. The assay is extremely specific for 6AM, thus eliminating interference from other opiates and their metabolites, which are often found at much higher levels than 6AM. The following cross-reactivities were found: Morphine (0.11%), Codeine (0.001%), Morphine 3-glucuronide (0.002%), Morphine 6-glucuronide (<0.001%), Hydrocodone (0.002%), and Oxycodone (0.002%).

A group of samples (n=80) containing a majority of 6AM positives by GC/MS were obtained from the Ventura County Correction System. All 74 of the samples positive for 6AM by GC/MS also tested positive by the CEDIA® 6AM assay, while all 6 GC/MS negative samples were below the CEDIA® 10 ng/mL cutoff. In a separate study, 3353 opiate negative urine samples were analyzed with the CEDIA® 6AM assay, none of these samples were found to be positive. Intra-assay precision studies yielded a standard deviation of 0.3 ng/mL at the 10-ng/mL cutoff.

Thus the CEDIA® 6AM assay is a convenient and effective method for the specific detection of 6AM, a unique indicator of heroin abuse. The assay can be applied to high throughput automated analyzers, and can improve efficiency of drug screening by eliminating the need for GC/MS confirmation of samples containing only morphine, prescription opiate drugs and their metabolites. Also, it can eliminate the defense raised by subjects testing positive in conventional opiate tests based on ingestion of foods containing poppy seeds.

Keywords: 6-monoacetylmorphine, screening, immunoassay

54 Evaluation of Six Screening Methods for Opiates in Urine

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In October 1998, the Federal Workplace Drug Testing Program changed urine screening and confirmation cutoff values for opiate testing from 300 to 2000 ng/mL-using morphine as the target compound. A 6-acetylmorphine (6AM) concentration ≥ 10 ng/mL was required to prove heroin use. To evaluate selected screening methods, we used six commercial immunoassays that employed either the 300 or 2000-ng/mL cutoff value to test 921 urine samples collected from 11 male human subjects following single doses of heroin. Subjects gave informed consent and stayed on a medically supervised, closed clinical ward. Eight received intravenous doses of 3, 6, and 12-mg heroin HCl and four smoked 3.5, 5.2, 10.5 or 13.9-mg doses of heroin. All urine specimens produced over a period of 3 days after dosing were collected, frozen, thawed and analyzed by the opiate immunoassays Roche ONLINE® 300/2000, Behring Emit II® 300/2000, Microgenics CEDIA® 300 and Abbott AxSYM® 300. Total morphine (Tmor) and 6AM concentrations were measured in each sample by GCMS, the confirmation method. The number of false positive (FP), false negative (FN), true positive (TP) and true negative (TN) results along with sensitivity, specificity and efficiency figures for each assay are listed in the table below. In the Morphine Only Method, samples that gave an immunoassay result ≥ screen cutoff were confirmed positive if the Tmor ≥ the same cutoff.

	MORPHINE ONLY METHOD						HEROIN METHOD		ROCHE EMIT		ROCHE EMIT CEDIA			
	AXSYM		ROCHE		EMIT		ROCHE	EMIT	CEDIA	AXSYM		2000	2000	
	300	300	300	300	2000	2000	300	300	300	300	FP	0	2	3
	5	39	1	11	24	203	206	251	161	FN	26	6	14	
	14	2	54	10	1	0	1	0	0	TP	40	60	241	
	241	253	201	29	38	41	40	41	41	TN	855	853	663	
	661	627	665	871	858	677	674	629	719					
SENSITIVITY(%)	60.6		90.9	94.5	94.5	99.2	78.8	74.4	97.4	100.0	97.6	100.0	100.0	
SPECIFICITY(%)	100.0		99.8	99.5	99.2	94.1	99.8	98.8	97.3	76.9	76.6	71.5	81.7	
EFFICIENCY(%)	97.2		99.1	98.2	97.9	95.5	94.0	97.7	97.3	78.0	77.5	72.7	82.5	

In the Heroin Method, these samples also had to have a 6AM $\geq$ 10 ng/mL to be confirmed positive. The Roche 2000 assay had poorer sensitivity and slightly better specificity than the EMIT 2000 assay for detection of morphine or heroin. For detection of heroin (ie. 6AM), changing to a cutoff of 2000 ng/mL markedly improved assay efficiency.

Key Words: immunoassay, opiates, urine drug testing

55 Variables Influencing Retention And Recovery Of Drugs On New Polymeric Mixed-Mode SPE Sorbents

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Quantitation of drugs and metabolites in sample matrices such as urine or plasma necessitates a sample clean up step such as solid-phase extraction (SPE) to remove endogenous interferences prior to analysis. New polymeric sorbents have been developed that contain both reversed-phase and ion exchange functional groups. The goal of this study is to gain an understanding of variables that influence the retention and recovery of analytes on these polymeric SPE sorbents.

The Oasis® MCX cation-exchange mixed-mode sorbent provides extremely clean extracts for basic drugs and allows for the fractionation of neutral and acidic drugs from basic drugs. Retention of neutral and acidic compounds is by a reversed-phase mechanism. Retention of basic compounds by a cation-exchange mechanism, because of its highly selective nature, allows the use of universal protocols. One protocol without a conditioning step gives high (>85%) and consistent recoveries (<5%RSDs) for a variety of basic drugs.

The Oasis® MAX anion-exchange mixed-mode sorbent can be used to provide extremely clean extracts for acidic drugs, and allows for the fractionation of neutral and basic drugs from acidic drugs.

Data will be presented illustrating the behavior of a variety of analytes (for example, amphetamine, methamphetamine, methadone, codeine, codeine glucuronide and naproxen) on the new sorbents. The information from this study results in the rapid development of simple, rugged and selective methods. A selective method for the extraction of amphetamine and methamphetamine gives recoveries >95% and RSD <9% by both HPLC and LC/MS analyses.

Key words: analysis of drugs in urine and plasma, solid-phase extraction, polymeric mixed-mode sorbents

56 Death due to Mirtazapine (Remeron) Toxicity.

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Mirtazapine (Remeron), is a novel antidepressant introduced onto the US market in early 1996, for the treatment of major depression.

A 24-year-old white male weighing 126 lb., with a history of drug abuse, was found unresponsive on his bedroom floor by his mother. Suffering from AIDS, he was extremely depressed according to his mother.

At autopsy, the findings were generally unremarkable,, however congestion was notable in both the brain and lungs.

Toxicological analysis for drugs of abuse, volatile and therapeutic drugs was performed. Mirtazapine was the only compound of significance detected. Mirtazapine was confirmed and quantified by capillary gas chromatography using nitrogen-phosphorus detection (GC/NPD) using a DB-17 column following a liquid / liquid extraction of mirtazapine from 1 mL of alkalinized matrix. After an acid back extraction, mirtazapine was re-extracted with methylene chloride. The extract was butyrated, evaporated and reconstituted in toluene for injection.

Mirtazapine was detected in whole blood at 2700 ng/mL, in urine at ~6500 ng/mL and in vitreous humor at ~2300 ng/mL. Death was ruled as an "Overdose of Mirtazapine".

Key Words: Mirtazapine, toxicity, postmortem

57 The Determination of Gamma HydroxyButyric Acid in Human Urine

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GHB or Gamma Hydroxybutyric Acid has been popularized as the "Sex Drug" of the 1990's. The small polar nature of the molecule and the lack of the presence of a UV chromatophore complicate the chromatographic and spectrophotometric analysis of GHB. Chemically, GHB is unstable and readily forms the GBH Lactone when heated under acidic conditions. Most analytical methods utilize this interconversion to determine GHB. This is problematic because GHB is manufactured from its lactone form. The following paper presents a method that utilizes SPE (new sorbent packing material-Clean Screen GHB) in combination with a liquid-liquid extraction and chemical derivatization to form the TMS derivative without the formation of the lactone form.

The sample is filtered by the use of a new sorbent material. The sorbent filters the interfering nitrogenous products of metabolism and allows GHB to pass through the sorbent. The GHB then can be isolated by evaporation and purified with a liquid-liquid extraction with DMF. The sample is evaporated and derivatized with BSTFA with 1% TMCS to form the Di-TMS derivative at room temperature. This derivative can be easily detected by GC-MS with SIM monitoring. The ions used for the determination were 233.1 (base ion) 234.1 (19%) and 235 (7%). The deuterated GHB-D6 was used as the internal standard with ions of 239.2 (base ion) 240.2 (20%) and 241.1 (9%).

Urea is also derivatized with BSTFA with 1% TMCS to form a Di-TMS derivative that has some of the same ions as GHB Di TMS including 147, 148, and 149; therefore the less abundant ions were used for the SIM analysis. The GHB-TMS derivatives are stable at room temperature for over 7 days.

The method is selective for GHB and not the GHB lactone however we can measure both of these analytes simultaneously. Another potential drug of abuse is the 1,4 butanediol, which is metabolically intercoverted to GHB. This method can also determine this new analyte.

The GHB method is validated from 1g/mL up to 100g/mL. Higher concentrations of GHB required the use of a smaller sample size. The recovery of GHB from human urine for this method was approximately 70%. The validation of this method showed %RSD of less than 7% for the linear range of the assay (1 to 100g/mL).

CONCLUSIONS: A new method for the determination of GHB in Human Urine is presented without the interconversion of GHB to its lactone form. This method is simple, easily accomplished and will quantitate the parent drug without the interference of the GHB Lactone. The validation of this method will be presented along with collaborative efforts to test clinical samples. If needed, both GHB Lactone and 1,4-butanediol can be determined with this method.

KEY WORDS: GHB, Analytical Methods, Date Rape Drugs

58 Death resulting from intravenous self-administration of benzonatate (Tessalon)

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Benzonatate (Tessalon), a medication structurally similar to the para-amino benzoic acid class of local anesthetics, is prescribed for the symptomatic relief of cough. While little information is currently available in the literature regarding therapeutic and toxic levels for benzonatate, information supplied by the manufacturer indicates that a single oral dose of 100 and 200 mg produces peak plasma concentrations of approximately 0.13 and 0.24 ug/mL, respectively (personnel communication, Forest Pharmaceuticals, Inc.). Here we report for the first time a fatality resulting from the intravenous administration of benzonatate.

A young woman discovered her 21-year-old husband unresponsive on the bathroom floor. In the sink, a syringe, spoon, lighter, belt, and two 100-mg benzonatate capsules were found. The capsules had been sliced open and the contents removed. The individual was quickly taken to the local hospital where he survived for 13 hours with assisted ventilation. Samples were submitted to our facility by the coroner for toxicological analysis. With the exception of benzonatate, no drugs or alcohol were found in the blood or urine specimens after screening using a combination of EMIT, Toxi-Lab and GC-NPD detection. Quantitative analysis of benzonatate was performed on blood and urine obtained upon admission to the hospital. Benzonatate (supplied by Forest Pharmaceuticals, Inc.) was utilized to produce a linear response curve for the method over the concentration range from 0.25 ug/mL – 10.0 ug/mL. Mepivicaine (internal standard) and n-butyl chloride were added to 1.0 mL of sample. The organic layer was extracted, evaporated to dryness and reconstituted in 35 uL of methanol. Analysis of 1 uL was performed by GC-NPD using a 15 m x 0.25 mm DB-17 column. The oven program began at 180^0C for one minute and then increased at 20^0C/min to 260^0C. The blood and urine concentrations were determined to be 0.8 and 52.0 ug/mL, respectively. It is not known whether the deceased had used benzonatate prior to the apparent fatal intravenous administration. A rinse of the syringe found in the bathroom of the deceased was found to be positive for benzonatate with no other drugs present. At autopsy, the primary pathological findings were cerebral edema and diffuse alveolar damage with superimposed bronchopneumonia. The pathologist determined the cause of death to be anoxic encephalopathy due to the injection of a foreign material.

Key words: Tessalon, benzonatate, overdose

59 Developing a Drugs Module For The National Coroners Information System.

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There are about 18,000 deaths a year in Australia that are referred to a Coroner. The National Coroners Information System (NCIS) formed in response to the 1994 Australian Coroners' Society endorsed recommendation that a national coronial database be developed. The aim of the NCIS project is to develop and maintain a high quality information service for coroners, which will also be of assistance to policymakers and researchers in the field of public health and safety. Currently there is no systematic way to collect coronial data on a national basis. The NCIS will be a national centralized data storage and retrieval system with data stored in fields, codes and text attachments.

The 'core dataset' for the NCIS will include information gathered from coroners' case files most of which is currently collected in each jurisdiction: victim demographics, cause of death details, incident information time and location of incident, textual information, police report autopsy report, toxicology report, Coroners finding and investigation information. In addition more detailed data relating to drug-related deaths will be incorporated in a drugs module.

Nationally standardised procedures and reports during coroners' investigations are crucial to developing the NCIS drugs module as a tool for defining and helping to resolve Australia's drug problems. The standardized approaches include the collection of initial scene and case demographic information by police, and postmortem procedures and reports. For toxicology this includes the type of specimen, scope of testing classification of levels of significance and of toxicological findings, drug group classification, as well as methods of analysis, units of measurement and route of drug administration. This will enable coroners, police, toxicologists, pathologists, researchers and policy makers to make informed judgements about how the information can be best utilized. The drugs module will provide early warning of changes and trends relating to harmful drugs. The NCIS will ultimately benefit the Australian community by contributing to a reduction in preventable death and injury- saving lives and money.

KeyWords: Drugs, Information, database

60 Effect of Implementation of 2000 ng/mL Opiate Cutoff for DHHS Regulated Specimens

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On Dec.1, 1998 all US Department of Health and Human Services (DHHS) certified laboratories implemented an opiate cutoff of 2000 ng/mL for the initial test and a 2000 ng/mL Gas Chromatography/Mass Spectrometry (GC/MS) confirmation for codeine and morphine. Additionally any specimen with a morphine concentration ≥2000 ng/mL requires a 6-acetyl-morphine (6-AM) analysis using a 10 ng/mL GC/MS cutoff. The cutoffs were previously 300 ng/mL for both the initial test and confirmation of codeine and morphine. This historical study was undertaken to assess the impact the above change would have on the laboratory and the number of positive opiate results reported to the MRO. Data from two groups of specimens were compared. Group 1 (n=645083) tested with the 300 ng/mL cutoff were received in the laboratory in Nov-April, of 1996-1997 and 1997-1998. Specimens from Group 1 reported as positive using a 300 ng/mL GC/MS confirmation cutoff were grouped according to concentration as follows: (1) specimens which only contained codeine ≥ 2000 ng/mL, (2) specimens which contained only morphine ≥ 2000 ng/mL , and (3) specimens which contained codeine and morphine ≥ 2000 ng/mL. These results were presented at the AAFS meeting in February, 1999 and predictions were made concerning the 2000 ng/mL cutoff. Group II (n=253,326) tested with the 2000 ng/mL cutoff were received in Dec 1998 through April 1999. The specimens from both groups were screened with Roche Diagnostic Systems Abuscreen ONLINE reagents at the correct cutoff and presumptive positive specimens were confirmed using GC/MS. The following statistics of the two groups respectively were; % presumptive positive all drugs, 3.70%, 2.91 %; presumptive positive opiates 0.86%, 0.32%; reported positive all drugs, 2.99%, 2.64%; reported positive opiates 0.40%, 0.23 %; reported positive 6-AM, not determined, 0.019%. DHHS guidelines require clients to send blind positive specimens for each drug. The blind specimens could not be eliminated from either group. The original data predicted 0.10% positive opiates, however, group II demonstrates 0.23%. After review of the data in-group II, one could attribute this discrepancy to blind specimens submitted by the clients.

Key words: Opiates, 6-Acetylmorphine and 2000 ng/mL cutoff

61 The Post-Mortem Distribution of Carbaryl in a Fatal Ingestion of Sevin.

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This case report is being presented due to the very high values for the distributed carbaryl in this suicidal ingestion of Sevin.

The deceased was a 48-year-old Oriental male who was found dead by the staff of the motel where he had spent his last night alive. He had been depressed over substantial debts and had expressed suicidal intentions. His disappearance on the night of his death had resulted in a missing person's alert.

Scene investigation revealed that the body was cool and in full rigor. A nearly empty 16 ounce container of Ortho Sevin liquid brand carbaryl insecticide concentrate was found on the table next to the bed. The stomach contents contained approximately 500 cc of a chalky material similar to that found in the bottle. Two large cans of beer (both opened - one empty and one full) were also found at the scene, but post-mortem analysis failed to reveal the presence of any ethanol.

Carbaryl was extracted from fluids and tissues by a simple acidic extraction into chloroform. The concentrated extract was submitted to SIM-GC/MS without derivatization. Mephobarbital was used as the internal standard. Two ions were monitored: 144 and 115. Because of the concentration in the specimens, multiple dilutions were required. The distribution of carbaryl was as follows: Blood (117 mg/L), urine (69 mg/L), liver (605 mg/Kg), brain (332 mg/Kg) and 93 grams were recovered from the stomach contents.

Keywords: Carbaryl, Sevin, Pesticide ingestion

62 Huffing: A Fatal Case of Chlorodifluoromethane (Freon 22) Inhalation

Nancy R. Haley, MS, Elizabeth A. Laposata, MD, Shirley H. Wick, MD, Paul S. Iwuc, MS, Laurie M. Ogilvie, MS, Theodore G. Pliakas, BS, RI Department of Health, Office of the Medical Examiner, Forensic Toxicology Laboratory, 50 Orms Street, Providence, RI* Huffing is the act of getting high from inhaling the toxic fumes of chemicals. This form of drug abuse involves the inhalation of intoxicating chemical vapors from common products such as: glues, nail polish remover, marking pens, spray paints, gasoline, butane, and fluorocarbons. This paper will describe a fatal case of huffing chlorodifluoromethane (Freon 22). Fluorocarbons comprise a group of synthetic halogen-substituted methane and ethane derivatives, which have commercial application as aerosol propellants and refrigerants. These fluorocarbons are capable of producing rapid death in persons abusing the chemicals for their euphoric effects.

J.M., a 28 year old male, was found unresponsive in a hotel room with a plastic bag enveloping his face and an adjacent Freon canister. He had checked in approximately 9 hours prior to his discovery. The canister was cold and condensation was present inside the plastic bag. The postmortem examination was conducted 24 hours after death. No anatomic cause of death was found. Fine white froth covered the larynx and trachea. Specimens of lung preserved in airtight crimp top glass vials and heart blood in rubber stoppered glass vials were collected. Toxicological evaluation revealed chlorodifluoromethane present in blood and lung tissues.

Exposure to fluorocarbons may result in death by direct toxicity to the myocardium, initiating a fatal arrthymia, and/or by the exclusion of oxygen from an enclosed space, such as a plastic bag, producing asphyxia. Thorough scene investigation is essential to direct the medical examiner and toxicologist to the correct specimen collection and analytical procedures and, ultimately, to the correct cause of death. The quantitation of chlorodifluoromethane in this case revealed levels below those previously reported in fatalities. This may be due to artefactual lowering of the levels due to evaporation of Freon 22 from the body during the postmortem interval or, more likely, indicates that asphyxia due to lack of oxygen in a limited space was the major mechanism of death. The cause of death in this case was certified as asphyxia due to hypoxia induced by the inhalation of Freon 22 in an enclosed space.

Key Words: huffing, inhalants, Freon 22

63 Cocaine Metabolite Elimination Patterns in Chronic Cocaine Users During Drug Cessation

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Previous reports suggest that creatinine is an appropriate analyte for normalization of drug concentration to reduce variability in concentration that is attributable to changes in urine volume. This study evaluated the effect of creatinine normalization of the semi-quantitative cocaine metabolite concentration in random urine specimens with creatinine concentration determined by a modified Jaffe method on a Hitachi 704 automated clinical analyzer. Another goal was to determine cocaine metabolite detection times following heavy cocaine use at 150, 100 and 30 ng/mL cutoffs assayed with the Abbott TD_X fluorescence polarization immunoassay (FPIA) on a TD_X instrument. Six healthy male subjects with a history of chronic cocaine use resided for 7-14 days on the closed research ward of the IRP, NIDA. All urine specimens were collected throughout the study, in the absence of drug administration, beginning immediately upon their entry to the ward. Subjects reported current cocaine use on a mean of 9.9 3.1 days in the past 2 weeks. Mean reported daily use was 1.1 0.7 g cocaine/day (range 0.12 to 2.5 g). The mean SEM creatinine concentration (N=298) was 91.9 3.2 mg/dL; a total of 9 specimens had urine creatinine concentrations less than 20 mg/dL. Peak urine cocaine metabolite (benzoylecgonine equivalents, BE) ranged from 5,893 to 68,575 ng/mL. The mean detection times for the first negative and last positive urine results for the 150 ng/mL and cutoff were 71.9 5.7 and 83.9 8.5 h and for the 100 ng/mL cutoff was 75.3 4.1 and 107.3 7.4 h, respectively. The detection time for the last positive result at the LOQ of the method (30 ng/mL) ranged from 119 to 235 h with a mean detection time of 173.2 20.3 h. Peak BE/creatinine ratios ranged from 14,736 to 60,686 ng/mg. Normalization of the BE concentration to the urine creatinine concentration improved the elimination curve and accounted for variation in individual states of hydration. Overall, these data indicate that heavy cocaine users have detection times in urine of only 3-4 days at 150 ng/mL and 4-5 days with a 100 ng/mL cutoff.

Key words : Creatinine, Benzoylecgonine,Urine, Detection time, Cocaine

64 Performance Evaluation of 4 On-Site Drug Testing Devices to Qualitatively Determine the Presence of the Drugs of Abuse

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We evaluated the performance of 4 on-site drug testing devices which utilize competitive binding immunoassays to qualitatively determine the presence of drug in urine: Triage Panel for Drugs of Abuse plus TCA, Quickscreen Pro-Multi Drug Screening Tests, Syva Rapid Test d.a.u. 5 and d.a.u. 2, and Rapid Drug Screen. All devices simultaneously determine the presence of the following drugs of abuse: amphetamines (AMP), benzoylecgonine (BE), ^{9}-tetrahydrocannabinoic acid (THCA), opiates (OPI), and phencyclidine (PCP). Triage also simultaneously tests for benzodiazepines (BZB) and barbiturates (BRB), whereas the other devices require separate panels for the BZB and BRB analyses. Two hundred twenty-two urine specimens containing drug concentrations around or above cut-off values were screened by ONLINE immunoassay. Of these, 199 yielded positive GC/MS results with at least 17 positive specimens in each drug class. Drug-added samples were also prepared at the following concentration: 33.3% below the cut-off, 16.7% below the cut-off, at the cut-off, 16.7% above the cut-off, 33.3% above the cut-off, and 66.7% above the cut-off. All specimens were then analyzed by all four on-site testing devices.

Urine Specimens			Drug Added Specimens		
	(%)	FN (%)		FP (%)	FN (%)
Triage	3.9	0.5	Triage	0.7	0.7
Quickscreen	6.2	2.4	Quickscreen	12.3	4.7
Syva	5.8	1.3	Syva	9.5	17.9
Rapid Drug	3.8	2.9	Rapid Drug	3.3	27.1

The evaluations around the cut-off concentrations revealed Triage Panel for Drugs of Abuse plus TCA with FP and FN rates of 0.7% to most reliably screen for the presence of drugs. Additionally, Triage required the least amount of test volume and had the most straightforward result assessment, making it, of the devices studied, the most dependable and reproducible on-site drug screening device.

Key Words: On-site drug tests, drugs of abuse, immunoassays

65 Detection of Cocaine and its Major Metabolites in Plasma and Urine Samples in an Emergency Medicine Setting Using Solid-Phase Extraction and GC-MS

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Cocaine (COC) is one of the leading illicit drugs in the United States approximately 20 million people in the USA use the drug. Since cocaine use is associated with increased cardiovascular and cerebrovascular events that can lead to sudden death, we evaluated the presence of COC and its major metabolites in 29 emergency department patients (18 males and 11 females, age 19 to 55) whose urine screened positive for benzoylecgonine (BE) using FPIA (Abbott AxSYM®). The following analytes were quantitated in EDTA plasma and urine (ng/mL): COC, BE, ecgonine methyl ester (EME) and norcocaine (NC). Deuterated internal standards (D_3 COC, D_3 BE, D_3 EME) were added to plasma or urine, along with 5N perchloric acid. Samples were vortexed and centrifuged to obtain a protein-free supernatant, which was added to a conditioned, solid-phase extraction column (Isolute® HCX 200 mg). Interfering substances were removed sequentially using water, 0.1N HCl, and methanol. A solution of methylene chloride:Isopropanol: ammonia (78:20:2, v/v/v) was used to obtain the final eluent that was evaporated to dryness under a stream of air. The residue was reconstituted in acetonitrile and transferred to autosampler vials. Extracts were derivatized using BSTFA with 1% TMCS at 60^0C for 30 minutes, and subsequently analyzed by GC-MS (HP 5973 MSD) in SIM mode.

The concentration range of plasma COC and its metabolites were: COC (16 - 130 ng/ml , n=3), EME (27 - 95, n=9), and BE (18 -1,390, n=22). All samples were negative for NC. In urine, the concentration range was: COC (3.6 - 40,130, n=23), EME (36 - 660,500, n=27), NC (8.5 - 2,520, n=9); all specimens were positive for BE (106 - 3,361,000, n=29). BE was the only metabolite present in two urine specimens (at concentrations of 407 and 435 ng/mL). Two patients had plasma and urine specimens positive for all analytes (except NC in plasma). The patient with the highest urine concentration of COC (40,130), EME (660,500), BE (3,361,000) and NC (2,520) had a small quantity of BE (465) in plasma. We conclude that this method simultaneously detects and quantitates COC and its major metabolites in plasma and urine, and shows 100% concordance with FPIA screening for detecting urinary BE.

Key Words: Cocaine, Cocaine metabolites ,GC-MS

66 Effects of Infusion Duration on Plasma Drug Concentrations Following Intravenous (IV) Cocaine Administration.

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Cocaine abuse occurs through different routes of administration which vary in toxicity and abuse liability. Drug delivery rate differences may explain some of the variation. Using a double blind, ascending dose, within-subject design, subjects received placebo or cocaine HCl in saline (10 mg, 25 mg, and 50 mg) at three IV infusion durations (10 sec, 30 sec, and 60 sec) over ten study sessions. Subjects (N=5) were healthy volunteers with a history of cocaine abuse. The study was approved by the NIDA Institutional Review

Board and each subject gave informed consent. During the study, all subjects resided on the NIDA IRP research ward, under continuous medical surveillance. Blood was collected 30 min prior to cocaine infusion and approximately 2.7, 5.5, 8.5, 16.5, 28.5, and 46.5 min post-infusion. Blood was refrigerated, centrifuged within two hours of collection, then the separated plasma was frozen at -20°C until analysis. Specimen preparation included SPE (CleanThru ZC-DAU020; UCT), derivatization with BSTFA (1% TMCS) and GC/MS analysis.

Generally, a higher mean peak plasma cocaine concentration was achieved with the shortest infusion duration (10 sec) for all three cocaine doses. There was considerable inter-subject variability at different doses/infusion rates. Since the first blood draw was at approximately 2.7 min, the cocaine peak concentrations may have been higher prior to this time. The peak plasma cocaine concentration (C_{max}) and time of peak cocaine concentration (T_{max}) are included for each dose and infusion duration in the following table.

Dose		10 mg			25 mg			50 mg		
Infusion Duration		10 sec	30 sec	60 sec	10 sec	30 sec	60 sec	10 sec	30 sec	60 sec
C_{max} (ng/mL)	Mean	116.7	106.8	83.2	297.1	199.3	232.6	434.4	362.6	387.5
	S.E.M	27.2	39.4	17.7	80.4	46.9	68.2	72.3	60.4	84.3
Range		38.8 - 197.9	33.5 - 228.2	39.7 - 146.4	79.4 - 547.2	71.2 - 318.0	87.8 - 479.9	212.2 - 645.7	198.9 - 571.7	156.4 - 618.1
T_{max} (min)	Mean	9.7	7.3	7.0	9.2	7.6	4.9	6.5	12.6	10.0
	S.E.M	4.9	2.9	2.5	4.8	2.5	1.5	2.6	4.6	4.6
Range		2.7 - 28.5	2.0 - 17.8	2.7 - 16.5	2.7 - 28.5	3.0 - 16.5	2.0 - 8.8	2.0 - 16.5	3.0 - 28.5	4.8 - 28.5

The data demonstrate a consistent relationship between cocaine dose and C_{max}, but no consistent relationship between C_{max} and infusion duration.

KeyWords: Cocaine, Infusion, Plasma, GC/MS

67 An OnLine II Two-Reagent Immunoassay for the Detection of Opiates 300/2000 on the Cobas Integra 700 and 400 Analyzers

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In order to comply with recent SAMHSA guidelines that raise the opiate screening cutoff from 300 ng/mL to 2000 ng/mL in urine, an Opiates OnLine II immunoassay has been developed. This new technology format is based on the kinetic inhibition of the agglutination reaction between antibody-coated microparticles and soluble drug-conjugate by free drug in the sample. The same formulation of reagents presented herein for the Cobas Integra 700 and 400 analyzers was previously applied to the Hitachi 717, 747, and 917 analyzers, and the Olympus AU800 and AU5200 analyzers. With the application of a two-reagent system on the Integra 700 and 400 analyzers, the instrument throughput has been increased to 600 tests per hour and 226 tests per hour, respectively.

One benefit of the Opiates 300/2000 assay is that the intra- and inter-assay precision over the range of the curve is less than 5% on all the above instruments. The intra-assay CV at the 300 ng/mL cutoff is 2.9% on the Integra 700 and 4.1% on the Integra 400, while the CV at the 2000 ng/mL cutoff is 2.2% and 1.8% on the Integra 700 and 400, respectively. The 2000 ng/mL cutoff assay offers a broad dynamic range with the ability to detect opiates from 0-8000 ng/mL. In addition, the OnLine II Opiates 300 ng/mL cutoff assay offers an increased dynamic range (0-2000 ng/mL) when compared to the previous opiates assay on the Integra analyzers. A method comparison of 50 positive clinical samples gave 100% agreement with GC-MS on both the Integra 700 and 400. The new opiates immunoassay has also been shown to be unaffected by the presence of common over-the-counter medications and to have structurally related cross-reactivities similar to the original OnLine assay.

STRUCTURALLY RELATED COMPOUNDS

COMPOUND	INTEGRA 700 APPROXIMATE CROSS REACTIVITY 2000 NG/ML CUTOFF	INTEGRA 700 APPROXIMATE CROSS REACTIVITY 300 NG/ML CUTOFF	INTEGRA 400 APPROXIMATE CROSS REACTIVITY 2000 NG/ML CUTOFF	INTEGRA 400 APPROXIMATE CROSS REACTIVITY 300 NG/ML CUTOFF
6-Acetylmorphine	79%	84%	89%	84%
Codeine	109%	107%	132%	129%
Dihydrocodeine	56%	60%	90%	93%
Diacetylmorphine	66%	65%	NA	NA
Dihydromorphine	48%	52%	51%	67%
Ethyl Morphine	73%	68%	92%	90%
Hydrocodone	32%	38%	33%	41%
Hydromorphone	21%	25%	35%	32%
Morphine-3-glucoronide	57%	63%	37%	42%
Thebaine	27%	30%	35%	39%
Meperidine	0.41%	0.38%	0.31%	0.48%
n-Norcodeine	2%	2%	0.36%	0.42%
Oxycodone	0.39%	0.41%	0.43%	0.64%

Key Words: Opiates, Online, Integra

68 A Case of Fetal Carbon Monoxide Poisoning

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A 35 week gestation female baby was delivered stillborn to a 20 year old Hispanic mother who had presented to the emergency room complaining of flu-like symptoms. The grandmother of the child was staying in the same motel room and later presented to the ER complaining of similar flu-like symptoms. Both the mother and grandmother were suffering from the toxic effects of carbon monoxide that had been released into the room via a faulty heating system. The mother and grandmother survived. The stillborn baby was sent to the Office of the Chief Medical Examiner for autopsy.

The autopsy, metabolic screening and toxicological screening for alcohol and drugs were all negative. Analysis of the baby's blood for the presence of carboxyhemoglobin was initially determined to be 61% spectrophotometrically using an IL 482 CO-Oximeter. Our confirmatory microdiffusion assay into palladium chloride, however, only indicated a "++", a reading consistent with 21 – 25 % carboxyhemoglobin saturation. Gas chromatographic analysis of the blood showed the carboxyhemoglobin saturation to be 27%. The cause of death was determined to be carbon monoxide toxicity and the manner of death, accident.

Fetal hemoglobin has been shown in a limited amount of clinical literature to cause falsely elevated carboxyhemoglobin values when spectrophotometric assays are used. A discussion of this phenomenon in connection with our case results will be presented.

Key Words: carboxyhemoglobin, fetal hemoglobin, co-oximeter

69 Confirmation of the NIDA 5 Using a Single SPE Sorbent and an Eight Solvent Rapid Trace System

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The scope of this project is to demonstrate excellent analytical recovery on the NIDA 5 using ISOLUTE Confirm HCX mixed-mode cartridges and the RapidTrace SPE Workstation equipped with a single reagent set. The benefit of such an experiment to the laboratory chemist is that only one SPE cartridge needs to be stocked eliminating potential confusion. Furthermore, various extractions may be run simultaneously which

will dramatically improve throughput. Advantages of using one reagent set is that equipment downtime for reagent draining and re-priming is eliminated and time is conserved in reagent preparation.

The NIDA 5 are drugs of abuse that are most commonly analyzed in forensic cases. These drugs include the opiates, THC, cocaine, PCP and amphetamines. It would be ideal if there were one universal method for extracting all drugs. This is not a realistic scenario as many drugs possess drastically different properties. In order for selectivity to prevail, extraction methods must be tailored to certain functionalities of molecules. It is possible, however, to use a mixed-mode SPE sorbent in combination with eight solvents to achieve highly selective and efficient extractions.

The analytical method used for the extractions is as follows:
Spike urine samples with methanolic solutions.
Perform extractions on RapidTrace with the following reagent set: Water, Methanol, 0.05M Phosphate Buffer (pH 6.0), 0.01M Hydrochloric Acid, 1.0M Ammonium Acetate Buffer (pH 8.0), Acetonitrile/Acetone/0.01M Hydrochloric Acid (15/15/70), Hexane/Ethyl Acetate/Acetone (50/40/10), and Methanol/Ammonia (98/2).
Add isotopically labelled internal standards to eluates.
Dry under nitrogen (TurboVap LV) reconstitute in appropriate solvent, and derivatize.
Analyze by GC-MS.

Results obtained using the eight solvent reagent set and mixed-mode sorbent are as follows:

Analyte	Benzoylecgonine	Amphetamine	Opiates	Phencyclidine (PCP)	THC
Recovery (%)	91	90	84-95	88	97

This experiment verifies that high recoveries of the NIDA 5 may be obtained using a single sorbent and eight solvent reagent set, thereby saving time and simplifying the extraction procedure. Also demonstrated was the preference for polar solvents, and the effectiveness and efficiency of extractions using dilute ionic solutions.

Key Words: solid phase extraction (SPE), automation, NIDA 5

70 Metabolic Production of Amphetamine Following Multidose Administration of Clobenzorex

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The interpretation of urine drug testing results can have important forensic and legal implications. Particularly, drugs that are metabolized to amphetamine and/or methamphetamine pose significant concerns. Clobenzorex is an anorectic drug that is metabolized to amphetamine. Clobenzorex was administered to 5 subjects. Each subject took 30 mg daily for seven days and individual urine samples were collected ad lib for 14 days starting on the first day that the drug was taken. Urine pH, specific gravity, and creatinine values were determined. Gas chromatography/mass spectrometry (GC/MS) was used to determine the excretion profile of amphetamine and clobenzorex using a standard procedure with additional monitoring of ions at m/z 91, 125, and 364 for the detection of clobenzorex. Peak concentrations of amphetamine were detected at 82 to 168 h postdose and ranged from approximately 2900 to 4700 ng/mL amphetamine. The use of a regioisomer (3-Cl-benzylamphetamine) as internal standard allowed for accurate quantitation of the parent drug. Peak concentrations of clobenzorex were detected at 50 to 123 h post dose and ranged from approximately 11 to 47 ng/ml clobenzorex. However, in many samples, clobenzorex was not detected at all. This analysis revealed that the metabolite, amphetamine, is present in much higher concentrations than the parent compound, clobenzorex, yet, even at peak amphetamine concentrations, the parent was not always detected (limit of detection 1 ng/mL). Thus, in the interpretation of amphetamine positive drug testing

results, the absence of clobenzorex in the urine sample does not exclude the possibility of its use.

Key Words: Clobenzorex, Amphetamine, Asenlix

71 The Effects of Gammahydroxybutyrate (GHB) Administration Alone and in Combination With Ethanol on General CNS Arousal in Rats as Measured Using the Acoustic Startle Paradigm.

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Objective: To compare the effects of GHB alone, ethanol alone and ethanol + GHB administered in combination on the magnitude of the acoustic startle reflex in rats. This reflex is an indication of the overall arousal of an animal and has been used as a model for studying CNS depressant effects. Methods: The acoustic startle reflex is elicited by a 40 msec burst of noise at 115dB. Test sessions consisted of 450 presentations of the noise burst at intervals of 20 seconds with a session duration of 2.5 hours. Following the presentation of 50 startle stimuli (i.e., 15-min pretreatment baseline), the rats were briefly removed from the startle chamber and were treated with saline, ethanol (0.25 or 0.50 g/kg), GHB (100, 200 or 400 mg/kg) or a combination of ethanol + GHB by intraperitoneal injection. The rats were then returned to the startle chamber and 400 startle stimuli were presented over the remainder of the test session (2.25 hours). An accelerometer was used to quantify the maximum cage movement (startle response) elicited by each noise burst. Data were analyzed by analysis of variance (ANOVA) with repeated measures. In all statistical comparisons, $p < 0.05$ was used as the criterion for significance.

Results: Control rats (saline treated) exhibited a slight decrease in startle amplitude over the 2.5 hour test session. This effect was attributed to within session habituation. At the low doses examined, ethanol treatment alone had little effect on startle amplitude. In contrast GHB treatment produced a marked reduction in startle amplitude; both the magnitude and duration of this effect were dose-dependent. Most importantly, ethanol co-treatment significantly enhanced the reduction in startle amplitude produced by GHB treatment.

Conclusions: The observation that ethanol potentiates the startle-reducing effects of GHB is consistent with the suspected GHB-ethanol interaction reported in humans. Future studies will focus on the mechanism underlying this GHB-ethanol interaction on the acoustic startle reflex in rats.

Keywords: GHB/Ethanol, Rats, Drug Interactions

72 Determination of GHB in Clinical and Postmortem Specimens

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A sensitive and selective assay for gamma hydroxybutyrate (GHB) was developed, which measures GHB itself without conversion to gamma butyrolactone, (GBL). GHB concentrations were subsequently measured in clinical and postmortem blood, urine and vitreous humor specimens. One mL of specimen was used, and diethylene glycol (DEG) was added as the internal standard. Cold 0.1M H2SO4 (250µL) was added, and the specimens were extracted twice with ethyl acetate (6mL). The combined ethyl acetate was evaporated under nitrogen at < 50 °C. BSTFA: 1% TMCS (30 µL) and acetonitrile (60µL) were added and the solution allowed to derivatize for 15 min at 70 °C. After cooling, 2µL of the derivatized sample were injected into a gas chromatograph/mass spectrometer (HP 5970). This was operated in the selected ion monitoring mode, and the following ions were monitored: GHB m/z 233, 204, 117, and DEG m/z 235, 103, 117. Calibration was linear over the range 1 - 200 mg/L GHB.

Blood (N=17), urine (N=12) and vitreous humor (N=8) were obtained from postmortem cases where the circumstances suggested that GHB had not been ingested. All blood specimens were positive for GHB,

with concentrations ranging from 3 - 107 mg/L (median 9.9); 10 urine specimens were positive, with concentrations ranging from 1.1 - 27.3 mg/L (median 5.4); and 3 vitreous humor specimens were positive, with concentrations ranging from 1.2 - 6.1 mg/L (median 1.5). This confirms that GHB is produced postmortem in blood and other specimens, and caution must therefore be taken in interpreting postmortem GHB concentrations.

In addition, we present data on five clinical cases which tested positive for GHB. GHB was detected in the blood of a sexual assault victim (3.2 mg/L), in the blood of two driving under the influence (DUI) cases (33 and 34 mg/L), and in the blood and urine of two non-fatal GHB overdose cases (blood 130 and 221 mg/L; urine 16,000 and 22,000 mg/L). The observed clinical symptoms ranged from confusion, disorientation, vomiting and nystagmus to ataxia, sinus bradycardia, unconsciousness and apnea.

Keywords: GHB, postmortem concentrations, clinical effects

73 GHB in Clinical and Postmortem Blood, Urine and Serum specimens – Making Sense of the Numbers.

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In recent years, gamma-hydroxybutyrate (GHB), an endogenous central nervous system depressant, has become a substance of use and abuse. In addition, gamma-butyrolactone (GBL) is also used as a GHB substitute. Once ingested, GBL is rapidly converted to GHB. The pharmacological effects of both GHB and GBL are similar and range from euphoria at low concentrations (less than 50 μg/mL) to marked central depression, sleep, coma and death.

Following the ingestion of GHB or GBL, the concentrations detected in blood and urine are frequently many hundreds or thousands of micrograms per milliliter. However in cases where a delay between ingestion and specimen collection has occurred only low concentrations of GHB may be present in blood and urine specimens. At low concentrations, interpretation of GHB findings is complicated by the presence of reported endogenous concentrations of GHB or GBL in both blood and urine.

This study investigated the concentrations of GHB in persons not using the drug. Specimens including fresh and stored clinical urine, blood and serum specimens and postmortem blood and urine specimens were analyzed to determine endogenous concentrations of GHB in these specimen types.

GHB and GBL were analyzed in biological specimens as total GBL by GC/MS-SIM following acidification and extraction of 1 mL of sample.

Specimen Type	n	Mean (μg/mL)	Median (μg/mL)	SD (μg/mL)	Range (μg/mL)
Clinical Urine	29	< 2	<2		0 – 1.1*
Postmortem Blood	73	18	14	16	1.0 – 93
Postmortem Urine	27	3.2	1.9	3.4	0.5 – 11

* Only two of 29 cases contained concentrations above 0.5μg /mL

The effects of preservatives on GHB formation in postmortem blood and stored antemortem specimens will also be presented.

Keywords: Gamma-hydroxybutyrate, GHB, Interpretation

74 Analysis of Gamma-Hydroxybutyrate (GHB) in Urine by Gas Chromatography/Mass Spectrometry

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During the 1990s, gamma-hydroxybutyrate (GHB) has become a popular drug of abuse that has been commonly associated with sexual assaults. As a result, there has been an increase in requests for GHB analysis. However, the analysis of GHB in biological fluids has been difficult partially due to a small body of reference literature. The present analytical methods used to extract GHB in biological fluids involve the conversion of GHB to gamma-butyrolactone (GBL) by acid catalysis. Recently, United Chemical Technologies, Inc. has developed an extraction method utilizing CLEAN SCREEN® ZSGHB020 extraction columns, which permits the direct isolation of GHB in urine without the formation of its associated lactone.

A simple method for the direct analysis of gamma-hydroxybutyrate (GHB) from human urine is presented. The method utilizes solid-phase extraction, liquid-liquid extraction, and silyl-derivatization (using BSTFA with 1% TMCS), followed by gas chromatographic-mass spectrometric analysis using d_6-GHB as the internal standard. The method was linear from 5 mg/L to 500 mg/L, coefficients of variation were less than 10%, and the conversion of GHB to GBL was not observed. Twenty-six urine specimens, previously analyzed by an existing method, were analyzed yielding concentrations of GHB ranging from 0-6100 mg/L. The results of the two methods were highly correlated.

The novelty of the method described here is the ability to discriminate between GHB and GBL. Further advantages of this method include: (1) small sample volume; (2) rapid extraction using technologies available to toxicology laboratories; and (3) detection of higher molecular weight ions compared to previous methods.

Key Words: Gamma-Hydroxybutyrate, GHB, Gas Chromatography/Mass Spectrometry

75 The Rapid Analysis of Biological Specimens for Gamma -Hydroxybutyrate

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The past few years have seen a dramatic rise in the abuse of gamma-hydroxybutyrate (GHB) in the United States; primarily due to GHB's euphoric and sedative properties and its use as a steroid alternative in gyms. More recently there has been an alarming increase in the use of GHB in crimes of drug-facilitated sexual assault.

A rapid and sensitive headspace procedure has been developed for the analysis of biofluids containing GHB. Two separate aliquots of a biological specimen were spiked with an gamma-methylene-gamma-butyrolactone internal standard solution. One of the aliquots was treated with concentrated sulfuric acid for cyclization of GHB to gamma-butyrolactone (GBL) while the other remained untreated. Both aliquots were extracted with methylene chloride and concentrated. Extracts were screened for GHB (as GBL) using automated headspace gas chromatography/flame ionization detection (GC/FID).

Qualitative findings were quantitated and confirmed in a manner similar to the GC/FID procedure with some modifications. d_6-GHB was added to the aliquot at a concentration approximating that of GHB as determined by the GC/FID screen. The extraction was as above with the conversion to GBL, but analysis was by full scan GC/MS(EI). Quantitation was performed by comparison of the area of the molecular ion for GHB as GBL (86 m/z) to that of the molecular ion for d_6-GHB as d_6-GBL (92 m/z).

Modifications of a published solid-phase extraction procedure ("A Solid Phase Extraction Method for The Determination of Gamma-Hydroxybutyrate (GHB) in Urine Without Conversion to Butyrolactone (GBL), United Chemical Technologies, Inc.) allowed for secondary confirmation of GHB in biological specimens. Direct insertion probe/mass spectrometry/mass spectrometry (DIP/MS/MS) without derivatization was used for the analysis in place of the procedure's BSTFA-TMS derivative formation and analysis by GC/MS.

This analytical procedure allows for the rapid detection of GHB in biofluids. This sensitive procedure has proven useful for the toxicological investigation of cases of drug-facilitated sexual assault.

Key Words: GHB, gamma-hydroxybutyrate, drug-facilitated sexual assault

76 Over-the-Counter Nutritional Supplements, Precursors of Testosterone and Nandrolone.

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A number of agents, precursors of potent testosterone (T) and synthetic steroid nandrolone, are now available on the nutritional supplements market:)^4 steroids such as 4-androsten-3,17-dione, 4-androsten-3$,17$-diol and 19-nor-4-androsten-3,17-dione; and)^5 steroids such as dehydroepiandrosterone (DHEA), 5-androsten-3,17-dione, 5-androsten-3$,17$-diol and 19-nor-5-androsten-3,17-dione. They are banned by the major athletic organizations throughout the world due to their profound anabolic effect. Human metabolism of)^5 steroids, primarily DHEA, versus)^4 steroids has been investigated.

Standard methodology of athletic steroid testing in urine was employed. When administered orally,)^4 steroids rapidly convert into the ultimate urinary metabolites, androsterosterone (A) and etiocholanolone (Ec), via T, which for most men may result in a positive test due to a brief increase of T to epitestosterone ratio (T/E) above the 6/1 cutoff. The entire steroid profile including T is amplified by two to three orders of magnitude. The 19-Nor version metabolizes via nandrolone and gives a strong positive test result with high concentrations of 19-norA and 19-norEc.

)^5 Steroids including DHEA metabolize into the)^4 steroid 4-androsten-3,17-dione causing T/E ratio to rise. However, a positive result is less likely because this transformation occurs only to a limited extent and only a minority of men with originally high T/E may test positive. Amplification of other steroids is less dramatic compared to)^4 analogues. The metabolic profile shows two distinctive kinetic patterns: rapid rise and fall of major steroid concentrations which corresponds to the first pass metabolism and protracted rise and subsequent slow fall of Ec and 5$-androstandiol. An alternative and perhaps more preferable metabolic pathway of DHEA leads to its isomer dehydroandrosterone (DHA) via 5-androsten-3,17-dione and to isomeric 5-androstendiols. The ultimate product of this route is Ec and the resulting drop of A to Ec ratio indicate)^5 steroid abuse. 19-Nor-5-androstendione in addition to common nandrolone metabolites, 19-norA and 19-norEc gives distinctive specific metabolites, 19-nor DHEA and 19-nor DHA.

Key Words:)^4 and)^5 anabolic steroids, nutritional supplements, metabolism, detection.

77 A Dual Derivatization GC/MS Method for the Simultaneous Assay of Methamphetamine, Cocaine, Codeine, Phencyclidine, Methadone, Cyclazocine and Metabolites.

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Numerous methods combining solid phase extraction (SPE) and GC-MS for the analysis of diverse matrices for basic drugs have been published, but a single assay for a broad range of basic drugs has not been available. These methods have been very effective for single drug classes and for compounds with relatively low volatility. However, inherent disadvantages include coelution of analytes with common m/z fragments, and for smaller, more volatile compounds, such as methamphetamine and amphetamine, erratic recoveries. These erratic recoveries have been due to evaporative losses incurred during extraction and derivatization processes and have resulted in unreliable quantitative data for these analytes. We devised a dual derivatization technique employing our previously published combined SPE/GC-MS method which appeared to stabilize methamphetamine and amphetamine during the extraction and derivatization processes and improved specificity for some analytes while maintaining good recovery and sensitivity for a variety of other basic drugs. Briefly, following SPE, a 20 L aliquot of MTBSTFA with 1% TBDMCS was

added to the eluate. The solution was evaporated under nitrogen at 40 C until dry and reconstituted in 20 L of acetonitrile. The sample was transferred to an autosampler vial, capped, and a second 20 L aliquot of MTBSTFA with 1% TBDMCS was added. This solution was then incubated at 80C for 15-20 min, a 20 L aliquot of BSTFA with 1% TMCS was added, and it was again incubated at 80C for 45 minutes. Following derivatization, samples were analyzed by GC/MS (HP-1 or Phenomenex ZB-1 capillary column) in selected ion monitoring mode. Internal standards employed in the analyses included D_{11}-methamphetamine, D_5-amphetamine, D_3-ecgonine methyl ester, D_3-cocaine, D_3-cocaethylene, D_3-benzoylecgonine, D_3-codeine, D_3-morphine, D_3-6-acetylmorphine, pentazocine, and D_5-phencyclididne. Methamphetamine, amphetamine, cocaine and 12 related analytes, phencyclidine, cyclazocine, methadone, codeine and 3 metabolites and 6-mono-acetyl morphine were assayed across a concentration range of 1.25-1000 ng/mL (or mg) in nine different drug-free matrices. Matrices examined included acetate buffer, plasma, urine, whole blood, meconium, saliva, sweat and hair. Calibration curves were linear for all analytes with correlation coefficients =0.98. Limits of detection and limits of quantitation were determined over a concentration range of 1.25-100 ng/mL (or mg) to optimize sensitivity and ranged from 0.5-5.0 ng/mL (or mg) and 1-10 ng/mL (or mg) respectively for all analytes in all matrices. We currently employ this assay in pharmacokinetic studies and conclude that it may have broad application in the forensic and workplace drug-testing arenas.

Keywords: GC/MS Assay, Biological Matrices, Cocaine and Opiates

78 Quantitative Analysis of Alprazolam and 2α-hydroxyalprazolam in Human Plasma Using Liquid Chromatography Electrospray Ionization MS/MS.

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A sensitive and specific electrospray ionization HPLC-MS/MS method has been developed for the quantitative determination of alprazolam (AL) and hydroxyalprazolam (OH-AL) in plasma. After the addition of deuterium labeled internal standards of AL and OH-AL, plasma samples were buffered to alkaline pH and extracted with toluene:methylene chloride (7:3). Dried extract residues were reconstituted in HPLC mobile phase and injected on to a reverse phase C18 HPLC column. The analytes were eluted isocratically at a flow rate of 250 L/min using a solvent composed of methanol/water (60/40) containing 0.1% formic acid. The analyses were performed using selected reaction monitoring. The assay was sensitive to 0.05 ng/mL for both the parent drug and metabolite and linear to 50 ng/mL. The intra-assay percent coefficient of variation (%CV) for AL at 2, 5 and 20 ng/mL were all equal to or less than 5.6. At these concentrations, all OH-AL intra-assay %CVs were < 8.4. The inter-assay variabilities for AL were 11.8 %CV, 8.7 %CV and 8.7 %CV at 2.0, 5.0 and 20.0 ng/mL, respectively. The OH-AL inter-assay variabilities were 9.6 %CV, 9.2 %CV and 7.8%CV at these concentrations, respectively. The assay accuracy was equal to or less than 6.6% for both analyses at the three concentrations. The method was used to quantify AL and OH-AL in plasma samples collected from 10 subjects who were administered a 1 mg oral dose of AL. The mean AL concentration peaked at 11.5 ng/mL one hour after the dose and AL was detectable for 48 hours. The mean OH-AL concentration peaked at 0.18 ng/mL four hours after the dose and was undetectable by 36 hours. Hydrolysis of the plasma samples with beta – glucuronidase (H. pomatia from Sigma Chemical Co.) had little effect on the detected AL concentrations, but increased OH-AL concentrations substantially. Plasma/blood ratios for AL and OH-AL exceeded 1 in the study samples.

Keywords: Alprazolam, OH-Alprazolam, ESI HPLC-MS/MS

79 **WITHDRAWN**

80 **The Quantitation of 2-Oxo-3-Hydroxy LSD, a Metabolite of Lysergic Acid Diethylamide (LSD) in Human Urine: Comparative Analysis Using Liquid Chromatography/Selected Ion Monitoring Mass Spectroscopy and Liquid Chromatography/Ion Trap Mass Spectroscopy.**

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This paper compares the potential forensic application of two sensitive and rapid procedures (Liquid Chromatography/Mass Spectroscopy (LC/MS) and Liquid Chromatography/Ion Trap Mass Spectrometry (LC/MS/MS)) for the detection and quantitation of 2-Oxo-3-Hydroxy LSD (OH-LSD) a major LSD metabolite. Urine specimens were spiked with the internal standards lysergic acid methylpropylamide (LAMPA) and 2-oxo-3-hydroxy LAMPA (OH-LAMPA) and extracted using a combination of liquid and solid phase extraction method. LSD and OH-LSD were quantitated using LC/MS in the selected ion monitoring (SIM) mode and LC/MS/MS in the ion trap mode. OH-LSD calibration curves for both procedures were linear over the concentration range 0-8000 pg/mL with correlation coefficients (r^2) greater than 0.99. The observed Limit of Detection (LOD) and Limit of Quantitation (LOQ) for OH-LSD in both procedures was 400 pg/mL. Sixty-eight human urine specimens that had previously been found to contain LSD by Gas Chromatography/Mass Spectrometry (GC/MS) were reanalyzed by both procedures for LSD and OH-LSD. These specimens contained a mean concentration of OH-LSD approximately 16 times higher than the LSD concentration. Since both LC methods produce similar results, either procedure can be readily adapted to OH-LSD analysis for use in high volume drug-testing laboratories.

Keywords: Lysergic acid diethylamide, LSD, Urine metabolite

81 **Testing for potential interferants in the Detection of 2-Oxo-3-Hydroxy LSD a metabolite of LSD in Human Urine Specimens**

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A major product of LSD metabolism, 2-oxo-3-hydroxy-LSD(OH-LSD), present in the urine of LSD users, has been previously demonstrated to be a better marker for identifying LSD use than targeting the parent drug, LSD. Specifically, OH-LSD analyzed using LC/MS has been reported to be present at concentrations 16 to 20 times greater than LSD. To further support use of this procedure for forensic purposes, a study of potential interferants in the LC/MS confirmation of OH-LSD was conducted. In order to assess the potential for compounds interfering with OH-LSD or the internal standard (OH-LAMPA) eighty five over the counter and prescription type drug standards were investigated as potential interferants. For the wide range of compounds studied, only one drug (ergonovine) was detected at the retention time of OH-LSD. However, the OH-LSD identity and qualifying ion ratios were outside of their acceptance range indicating that this compound would not produce a false positive with this confirmation procedure. Additionally, one hundred specimens that gave false positive results when screened for LSD using the Syva EMIT II LSD immunoassay were analyzed by High Performance Liquid Chromatography (HPLC) on the BioRad Remedi instrument to identify the cross-reacting compounds. LC/MS analysis of these specimens demonstrated that none of the cross-reacting drugs and/or their metabolites interfered with the detection of OH-LSD or OH-LAMPA.

Keywords: Lysergic acid diethylamide, LSD, Urine metabolite

82 Investigation of Postmortem Drug (Nortriptyline) Redistribution Using a Rabbit Animal Model

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Fatal overdoses of tricyclic antidepressants are common encounters at ME offices. Studies have shown that the post-mortem concentrations of these and some other drugs vary with the site of origin of the sample as well as with the interval from the time of death. Besides the tendency of drugs to redistribute from sites of high concentration (tissues) into sites of lower concentration (blood), post-mortem temporal blood movement takes place. Fallani describes four stages of this movement. Thus, the post-mortem blood concentration of such drugs might not reflect the true blood concentration at the time of death. This phenomenon presents a serious challenge in interpreting post-mortem drug concentrations. We, herein, use a rabbit animal model: i) to determine the optimal dose of nortriptyline required to produce detectable concentration in vitreous humor; and ii) to define the relationship between postmortem concentration in the vitreous humor and that in the blood.

New Zealand white rabbits were used for this study. Each rabbit was injected with 2.5mg/kg nortriptyline in the marginal ear vein. Biofluids (blood, vitreous humor, urine) and various organs were collected immediately following the sacrifice of rabbits at 0, 0.5, 2.5, 6, 24 hours. Samples were kept frozen until time of analysis. Nortriptyline was quantitated using solid phase extraction and reverse-phase HPLC with UV detection at 245nm.

Nortriptyline Concentration (ng/ml) in Plasma & Vitreous Humor

Time (hours)	**0**	**0.5**	**2.5**	**6**	**24**
Plasma/serum	0	223	74	13	0
Vitreous Humor	0	29	15	0	0

Based on these preliminary results, we conclude that: 1) Rabbits could tolerate 2.5 mg/Kg nortriptyline. 2) Nortriptyline can diffuse readily into the vitreous humor of rabbits as early as 30 minutes. These results will give insight into designing protocols to address the following issue: how would postmortem drug concentrations in the vitreous humor reasonably reflect blood concentration at the time of death?

Key words: Post-mortem drug redistribution, Tricyclic antidepressants, Vitreous humor

83 Pharmacogentics For Rational Drug Therapy and Understanding Toxcity - Genotyping CYP2D6 for Antidepressant Therapy

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Most drugs are metabolized by Cytochrome P450 (CYP) mixed-function monooxygenases. More than 30 different forms of CYP have been characterized in humans. They are classified into 4 families (l-4) and subfamilies (A-E). The extent to which a drug is metabolized is partially determined by the genetic variation (polymorphism) of CYP. This may be characterized by the patient's response (phenotype) to the drug. Individuals lacking or having a reduced capability of metabolizing a drug are at an increased risk of developing adverse side effects. They are known as poor metabolizers (PM) and are homozygous for the mutation. On the other hand, individuals with unaltered capacity for metabolizing a drug are known as extensive metabolizers (EM). They carry the wild type allele. Ultraextensive metabolizers (UEM) having an

increased capability of metabolizing a drug have also been identified. Those have duplicate copy of the gene encoding the drug metabolizing enzyme. Conventional drug doses are subtherapeutic in these individuals. CYP2D6 is of particular interest since it is involved in the metabolism of tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSR1) (Fijordside L, et al. Pharmacogenetics. 9:55-60, 1999) and other drugs.

The objective of this study was to optimize the genotyping technique for the most common mutations of CYP 2D6 - 2D6*3 and 2D6*4 (Bon, M.A.M. et al. Clin Chem., 44:A98, 1998), with the potential application to our psychiatric patients who were on TCA therapy. We used polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis. First, DNA was isolated from peripheral blood using Puregene DNA isolation kit (Gentra Systems). Specific sequences of CYP 2D6*3 and 2D6*4 mutant genes were amplified by PCR. The amplified products were then digested using the restriction endonucleases, MvaI and MspI (Boehringer Mannheim). The length of the resulting fragments for each mutation was then analyzed using electrophoresis and ethidium bromide staining. PMs carrying 2D6*4 mutation were identified by the presence of a single 355 bp band. PMs carrying 2D6*3 were identified by the presence of 168, 82, and 20 bp fragments. This rationale may be useful in prescribing the appropriate drug and the dose before initiation of therapy, predicting patient's response to an antidepressant such as TCA or SSRI, and understanding potential drug interaction and toxicity.

Key words: Pharmacogenetics, genotyping, CYP2D6, antidepressant.

84 Conversion of 11-nor-^{9}-THC-9-COOH in the Presence of Pyridinium Chlorochromate Causing Failures in GC/MS Analysis of Urine Samples

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Recently, a number of urine samples were screened positive for THC by CEDIA® immunoassay method (Microgenics, Pleasanton, CA), while GC/MS confirmation analysis indicated no detectable levels of 11-nor-^{9}-THC-9-COOH. The GC/MS analysis had valid 11-nor-^{9}-THC-9-COOH-D_3 internal standard from extracted samples, so these samples were classified as false positive. Isolation of immunoreactive compound(s) from the samples was initiated using immunoaffinity extraction. The affinity extraction resin used an 11-nor-^{9}-THC-9-COOH specific monoclonal antibody (Microgenics, Pleasanton, CA) attached to agarose (Sepharose® 4B) resin. Full scan GC/MS analysis of immunoaffinity extracts after derivatization with N-methyl-N-(*t*-butyldimethylsilyl-trifluoroacetamide) [MtBSTFA] indicated one compound with a retention time of 10.69 minutes with a molecular ion of 568. By comparison, analysis of 11-nor--^{9}-THC-9-COOH was detected at 9.66 minutes with a molecular ion of 572. Both the THC acid metabolite and the unidentified compound produced common ions of 207, 281 and 355, indicating structural similarities. Speculation focused on the idea that 11-nor-^{9}-THC-9-COOH was converted through an oxidation process to the unknown material, which could still produce a detectable immunoassay response. Information about the adulterant "UrineLuck" indicated pyridinium chlorochromate [PCC] as an active ingredient, which could induce the proposed oxidation. Experiments verified that negative urine samples spiked with 11-nor-^{9} THC-9-COOH and PCC incubated > 24 hours showed conversion of the THC acid metabolite to the compound recovered from affinity extraction, having a molecular ion of 568 and the same mass spectrum. The mass-spectrum for this unidentified compound is consistent with cannabinol-9-COOH. Addition of deuterated internal standard immediately prior to extraction produced valid 11-nor-^{9}-THC-9-COOH-D_3 peak detection. However, PCC tainted urine samples spiked with deuterated internal standard and incubated > 24 hours produced similar conversion, verified by GC/MS. Further testing of the original samples also confirmed significant chromate levels. Despite the presence of an adulterant, the CEDIA-THC immunoassay correctly identified these samples from individuals that consumed marijuana. Modification of existing GC/MS-SIM can be implemented to identify samples containing cannabinol-9-COOH as supporting evidence for THC abuse masked by PCC adulteration.

85 Pharmacological Facilitation of Robbery: Analysis of Two Cases from the Emergency Department

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There are a variety of drugs that can be used for sedation to facilitate crimes such as robbery or rape.The most common are the benzodiazepine sedative hypnotics and GHB. The effects of these wane within 12 hours and most victims of such drugging do not seek medical help.

We report two cases of robbery associated with poisoning that resulted in prolonged sedation, amnesia and GI distress. Two male patients presented to the ED 24 hours after unknowingly ingesting a substance placed in their cocktail which rendered them unconsciousness for approximately 12 hours. Upon awakening, both patients experienced severe nausea and vomiting, abdominal cramping, myalgias and had total amnesia of the previous evenings events. Both patients subsequently slept for the next 12 hours then sought medical care through the ED. On presentation, both had stable vital signs, and were lethargic. Physical exam was unremarkable except for diffuse abdominal tenderness. Routine metabolic panel results were within normal limits. Later interviews discovered that each had items missing from their person and from their hotel rooms. Blood samples taken 24 hours after onset of symptoms were frozen and shipped on dry ice for analysis. Screening for GHB was negative, and GC/MS analysis revealed lorazepam (20 ng/ml) in one of the patient samples. The etiology of pharmacological sedation in the presence and absence of toxicological findings will be discussed based on clinical findings and in light of other cases.

Key Words: poisoning, robbery, benzodiazepines

86 CNS Catastrophes Presenting as a Common Adverse Event to Acute Cocaine Use

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In the cocaine abusing population presenting to our emergency department, CNS catastrophes are the second most common cocaine adverse event. We describe the clinical findings, outcome, and where available, blood concentrations of cocaine and its metabolites in 10 patients with cocaine associated CNS catastrophes. Five patients were male, 5 were female. The mean age was 39.8 ± 6.3 years. Three patients presented with focal neurologic deficits, 6 with change in mental status, and 1 with seizures. Three patients had strokes, 7 had intracerebral bleeding. Two patients died. All others had some type of permanent neurological deficit.

Cocaine and its major metabolite concentrations were measured in 4 patients by an extractive alkylation/mass spectrometry procedure. Six patients had positive urine drug screens for cocaine. Initial mean concentrations (mg/L) were as follows: cocaine 0.04±0.01, ecgonine methylester 0.02±0.03, cocaethlyene 0.15±0.02, ecgonine 0.25±0.13, norcocaine 0, and benzoylecgonine 0.40±0.53. Cocaethylene was not detected in all patients, but when present, was at higher concentrations than cocaine, most likely explained by its longer half life. These drug and metabolite concentrations are similar to what has been previously reported in the drug abusing population and do not predict CNS catastrophe.

Clinical evidence of stroke or ICH include seizures, decreases in mental status, unequal pupils and focal neurologic deficits such as unilateral motor or sensory loss. Evidence of these clinical findings are important as initial toxicological findings may be unimpressive and history may be unobtainable.

Key Words: intracerebral hemorrhage, cocaine abuse, stroke

87 Performance of 5 Non-Instrumented Drug Tests with Challenging Near Cut-off Specimens

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We have evaluated 5 non-instrumented drug testing devices with clinical urine specimens selected to be challenging. Sixty clinical specimens for each of the 5 SAMHSA-specified drug categories were selected such that approximately 10 specimens were clean negatives, 20 below, 20 slightly above, and 10 well above the immunoassay screening cut-offs. The tests were performed by both a scientist and a non-scientist with each result read by both. In addition, all specimens were tested on a small automated benchtop immunoassay analyzer. All specimens (298) were analyzed by GC/MS, and device performance was assessed based on GC/MS confirmability using standard cut-offs. The devices had an overall accuracy of 70% for the 2980 results, comparable to the automated analyzer (80%). There was also little difference in performance between the scientist and non-scientist.

	Sensitivity	Specificity	PPV	NPV	Accuracy
Syva RapidTest	83%	66%	.68	.82	74%
Syva RapidCup	95%	41%	.58	.90	66%
Roche TesTcup	89%	58%	.65	.86	73%
Biosite Triage	32%	98%	.93	.62	67%
Casco-Nerl microLINE	72%	67%	.66	.74	70%
Syva ETS analyzer	81%	79%	.77	.82	80%

Key Words: Non-Instrumented Drug Tests, On-Site Drug Testing, Urine Drug Testing

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