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S1

Application of Two-dimensional Gas Chromatography to the Detection of 11-nor-Δ^9-tetra-hydrocannabinol-9-carboxylic acid (THC-COOH) in Hair

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The proposed Federal regulations for the detection in hair, of 11-nor Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), a metabolite of marijuana, require a confirmatory detection level of 0.05 pg/mg. At present, the only way to achieve this on a routine basis, has been with the use gas chromatography with tandem mass spectrometry (GC/MS/MS) technology. Tandem mass spectrometry is an expensive approach and dissuades laboratories from attempting to enter the hair testing market.

A procedure for the determination of THC-COOH in hair using two-dimensional gas chromatography (GC x GC) coupled to mass spectrometry (GC/GC/MS) is described for the first time. The method makes use of several small improvements in the extraction, gas chromatography and mass spectrometry procedures to allow the required sensitivity to be achieved.

The results of this approach demonstrate detection of THC-COOH in hair at a concentration level of 0.05 pg/mg with both a target quantitation ion and a unique confirming qualifier ion, using a single quadrupole mass selective detector. These two ions and the enhanced separation of the GC/GC provide a high degree of confidence in the determinations. Hair specimens which had been previously analyzed using gas chromatography with triple quadrupole mass spectrometric detection were treated according to this procedure.

The method has been successfully applied to the detection of THC-COOH in hair specimens from known marijuana users, and reaches the levels currently proposed in the Federal Register.

Key Words: Marijuana, Hair, Two-dimensional GC

S2

Detection of Carboxy-THC in Hair Using a Novel Nanoscale LC/MS/MS Technique

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Attendees will learn a new technique for the identification and quantitation of the carboxy-tetrahydrocannabinol metabolite in hair. This presentation will impact the forensic community and/or humanity by exposing the attendees to a new and highly sensitive technique in liquid chromatography / mass spectrometry (LCMS) for the detection of c-THC in hair using nanoscale chromatography/ionization and ion trap MS/MS analysis.

The currently proposed SAMHSA (Substance Abuse and Mental Health Services Administration) limit of detection for screening c-THC is 0.05 pg/mg in specimens of sweat, saliva, urine, and hair, which is achievable in gas chromatography / mass spectrometry (GCMS) by derivatization (PFPA - pentafluoropropionic anhydride, for example) with analysis run-times typically more than ten minutes. To decrease sample preparation time and increase throughput, analysis by LCMS is attractive.

The advantage of analyzing hair for drugs of abuse is that it is more effective than urinalysis and other methods in correctly identifying drug abusers. Hair testing provides longer detection periods (approximately 90 days) and is particularly well suited and effective in pre-employment and random testing. It is non-intrusive and non-evasive. The shorter detection periods available with urine or saliva are effective with post accident and reasonable suspicion testing. Testing of hair is becoming more common and the fact that SAMHSA now considers hair to be a viable medium for drugs analysis further indicates this trend.

This work will show the comparison of present analytical methods using GCMS with ion trap mass spectrometry and a novel chromatography / ionization source known as HPLC-Chip/MS, where HPLC stands for high-performance liquid chromatography and the Chip is a new medium for providing both the sample chromatography and electro-spray ionization at only few hundred nano-liters per minute flow-rates.

A real sample from a known THC user is analyzed and quantified for the presence of c-THC with the deuterated D9 analog used as an internal standard. Current results show that a limit of detection (LOD) at the 250fg level on-column is achievable. This result provides a competitive alternative to standard GCMS techniques.

Key Words: LCMS, Ion Trap, THC, drugs of abuse

S3

Methamphetamine and Metabolites in Hair, Oral Fluid and Urine

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Federal guidelines for the potential use of hair, sweat and oral fluid as well as urine for workplace drug testing were published in the Federal Register in 2004. This study was designed to determine which of these specimens provided the most accurate information both in a population admitting to the use of methamphetamine, and a population denying methamphetamine use. Sweat was not included due to the difficulties of re-collection of the sweat patch after a few days.

The study enrolled 200 subjects, half of whom admitted to methamphetamine use, half who did not. Each subject provided a urine sample, an oral fluid and a hair specimen taken from the head at the time of interview. Information on drug use, including time of last use, frequency of use, ethnicity, age, sex and hair color were recorded for each subject. The hair specimens were analyzed for methamphetamine, amphetamine, 3-4 methylenedioxy-methamphetamine (MDMA), its metabolite methylenedioxy-amphetamine (MDA) and methylenedioxy ethylamphetamine (MDEA). The oral fluid specimens were confirmed for the presence of methamphetamine, amphetamine, MDMA and MDA.

When specimens were analyzed according to the levels proposed in the Federal guidelines for alternative samples, hair identified the highest number of drug users in both the admitted using population and those who denied use. Oral fluid and urine gave similar positivity rates. It was difficult to assess the affect of hair color on positivity rate or drug concentrations since the majority of subjects had dark hair. Overall, whites accounted for the highest number of positives in all specimen types, suggesting that positive rates, in this geographical area, may be related more to drug use patterns, rather than race or method bias.

While the analysis of amphetamines in hair, oral fluid and urine has been previously published, this is the first study where all three matrices were collected simultaneously from a drug using population, and analyzed according to the proposed Federal guidelines.

Key Words: Hair, Saliva, Amphetamines

S4

THCA Detection in Oral Fluid down to 10 pg/mL

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An objective of this study was to analyze oral fluid samples for the presence of 11-nor-Δ^9 -tetrahydrocannabinol carboxylic acid (THCA). An analytical method has recently been validated for detection of THCA in oral fluid at concentrations from 10 pg/mL to 240 pg/mL. This abstract will summarize method performance over the last year.

Evaluation of oral fluid for THCA was performed on samples determined to have 11-nor-Δ^9-tetrahydrocannabinol (THC) in their oral fluid. Subjects that screened positive by enzyme linked methods to cross reactive forms of THC and oral fluid that screened negative were also tested for THCA. Batches of samples were assayed blind for THCA prepared by a third party. Additional samples were tested for groups yet to be identified. The summary here includes method performance over the course of these studies. Stability of authentic samples, spiked samples and standards has been assessed.

This new method uses solid phase extraction of oral fluid samples followed by derivatization with HFIP and PFAA. Samples are injected on a GC/MS/MS TSQ7000 specially tuned for high sensitivity. Chemical ionization using ammonia gas is performed prior to separation and negative ion detection of 620 m/z parent mass and 492 m/z and 383 m/z fragments with internal standard detection of 622 m/z parent mass and fragments of 495 m/z and 386 m/z. Quantification is performed using the ratio of the 492 m/z drug and 495 m/z internal standard (THCA-d3).

Results indicate the new method used for this determination is more sensitive than several methods currently used; providing a tool for better detection of the presence of the major cannabis metabolite in the oral fluid. The method has been in production over the last year and has proved to be very robust. The results obtained for separate batches over the course of several weeks were within 20% relative standard deviation at the method limit of quantitation (LOQ).

Authentic samples of oral fluid have been tested. This includes over 400 samples from various sources and at least 31 batches of controls extracted and injected separately. Twenty seven batches were selected to calculate the following statistics: Low control average response; 11.2 pg/mL (target 10 pg/mL), standard deviation 1.864 (RSD=16.6%); High control average response; 54.79 pg/mL (target 60 pg/mL), standard deviation 5.14 (RSD=9.38%). Results of authentic sample stability, spiked sample stability, and standard controls are variable and will be further discussed when more data is generated and evaluated.

Conclusions: Method performance is adequate for forensic determination of THCA in oral fluid down to 10 pg/mL. THCA is detectable in oral fluid of marijuana smokers tested over the last year.

Key Words: THCA, Oral Fluid, Marijuana

S5

Cocaine and Metabolites in Hair, Oral Fluid and Urine

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Federal guidelines for the potential use of hair, sweat, and oral fluid as well as urine for workplace drug testing were published in the Federal Register in 2004. This study was designed to determine the positivity rate in various specimen types, both in a drug using population and a population denying cocaine use. Sweat was not included due to the difficulties of re-collection of the sweat patch after a few days.

The study enrolled 200 subjects, half of whom admitted to cocaine use, half who did not. Each subject provided a urine sample, an oral fluid and a hair specimen taken from the head at the time of interview. Information on drug use, including time of last use, frequency of use, ethnicity, age, sex and hair color were recorded for each subject.

The specimens were analyzed for cocaine and/or its metabolites depending on the matrix and the data is presented. When specimens were analyzed according to the levels proposed in the Federal guidelines for alternative samples, hair identified the highest number of drug users in both the admitted using population and those who denied use. Oral fluid and urine gave similar detection rates in both populations, with oral fluid slightly better in the non-using population, and urine slightly better in the self-reported users. However, oral fluid does not have the sample collection problems associated with urine.

It was difficult to assess the affect of hair color on positivity rate or drug concentrations since the majority of subjects had dark hair. Overall, blacks accounted for the highest number of positives in all specimen types, suggesting that positive rates, in this particular geographical area, may be related more to drug use patterns, rather than race or method bias.

While the analysis of cocaine and its metabolites in hair, oral fluid and urine has been previously published, this is the first study where all three matrices were collected simultaneously from a drug using population, and analyzed according to the proposed Federal guidelines.

Key Words: Hair, Saliva, Cocaine

S6

Flunitrazepam in Doping and Robbery Cases in the UK

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Flunitrazepam (Rohypnol) has achieved notoriety in drug facilitated sexual assaults, but despite much publicity it has been encountered in only very few cases in the UK. This presentation will cover a series of three cases involving the administration of flunitrazepam (FNZ) by a female with the intent to sedate male victims prior to robbery, the first such convictions in the UK. In case 1, GC-MS analysis on a urine sample from the victim showed the presence of approximately 1.3 µg/ml 7-amino-FNZ and 0.1 µg/mL 7-acetamido-FNZ. Approximately 1 ng/mL FNZ was also indicated. In case 2, the urine sample contained approximately 0.1 µg/mL 7-amino-FNZ and 9 ng/mL 7-acetamido-FNZ. The blood sample contained approximately 1 ng/mL FNZ. In case 3, the urine sample contained approximately 10 ng/mL 7-amino-FNZ with an indication of approximately 2 ng/mL 7-acetamido-FNZ. Approximately 0.1 ng/mL FNZ was indicated in the blood. A sample of wine recovered from the scene also contained FNZ. In case 2, the samples were not submitted until over two years after sampling, and the positive findings suggest that under adequate storage conditions FNZ and its metabolites appear to be relatively stable in blood and urine. An ongoing study is being carried out to determine the approximate detection times and stability of the metabolites in urine. Six volunteers took 1 mg FNZ and supplied preserved and unpreserved urine at successive time intervals. Maximum concentrations of 7-amino FNZ ranged between 10-75 ng/mL after 12-24 hours and it remained detectable in all samples at levels above 1 ng/mL for four days after consumption. Much lower levels of 7-acetamido FNZ were present; the maximum urine concentration ranged between 0.5 - 4 ng/mL after 12-36 hours and declined to below 2 ng/mL after 24 - 48 hours. A decline of between 10 - 50% of the initial 7-amino FNZ concentration was observed in selected preserved and unpreserved urine samples stored at 4°C for five months.

Key Words: Flunitrazepam, Doping, Stability

S7

Detection of Hydromorphone in Chronic Pain Patients with Correspondingly High Concentrations of Morphine in Urine

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A group of chronic pain patients (N=13) who were treated with high-dose morphine and had no personal or family history of addiction was studied. Initial evaluation and follow up of these outpatients with random urine drug tests excluded opioid misuse. The pain patients were chronically treated with morphine and other opioids (methadone, oxycodone and fentanyl). Urine specimens were collected and analyzed by GC-MS for the presence of hydromorphone at a reporting limit of 100 ng/mL. Ten of the 13 morphine-treated patients excreted hydromorphone in minor amounts ranging from 120 to 1400 ng/mL. Concurrent morphine concentrations were exceptionally high in the 10 patients and frequently exceeded the upper limit of linearity (> 10,000 ng/mL) of the assay. The ratio of hydromorphone to morphine was very low. Morphine concentrations in the 3 patients in which hydromorphone was not detected tended to be lower than those noted in other patients. The results demonstrate that hydromorphone can be produced as a minor metabolite of morphine in humans. This is similar to the minor metabolic pathway shown to exist for the conversion of codeine to hydrocodone. It is suggested that interpretation of low urinary concentrations of hydromorphone in combination with high concentrations of morphine in morphine-treated pain patients be done with caution. The presence of hydromorphone, therefore, should not be considered as conclusive evidence of hydromorphone misuse.

Key Words: Hydromorphone, Morphine, Metabolism

S8

Evaluation of Paired Urine and Oral Fluid Test Results from a Workplace Population

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The use of laboratory based oral fluid testing has seen a significant increase in recent years, however very few studies involving paired urine/oral fluid are available for review. In order to evaluate the reliability of laboratory based oral fluid testing, a customer implemented a random, blind testing program in which both urine and oral fluid samples were collected from donors in a workplace setting. Testing was conducted using Non-DOT donors subject to the Company's pre-employment, random, post-accident and reasonable cause testing program. Urine specimens were collected in accordance with HHS guidelines and tested in a SAMHSA certified laboratory. Oral fluid samples were collected using the Intercept™ Oral Fluid Collection Device and tested at another SAMHSA certified laboratory that has significant experience with the analysis of oral fluid specimens.

Of the 297 paired specimens tested, 286 screened negative in both the urine and oral fluid specimens. Of the remaining 11 non-negative specimens, 3 specimens were positive for benzoylecgonine in the urine specimen and positive for cocaine in the oral fluid specimen. One specimen was positive for benzoylecgonine in the urine specimen, positive for cocaine in the oral fluid specimen; and was also positive for cannabinoids in both the urine and oral fluid specimen. One specimen was positive for benzoylecgonine in the urine specimen, positive for cocaine in the oral fluid specimens and was also positive for opiates in both the urine and oral fluid specimen, although the opiates detected in the urine specimen were different than the opiates detected in the oral fluid specimen. Two specimens were positive for benzoylecgonine in the urine specimens and negative for cocaine in the oral fluid specimens although both oral fluid samples showed an elevated immunoassay result for cocaine. Three specimens tested positive for cannabinoids in the urine specimen, but showed no immunoassay activity for cannabinoids in the oral fluid specimens. One urine sample was positive for benzodiazepines; however the oral fluid sample was not tested for benzodiazepines.

In conclusion, this study suggests that the use of oral fluids for the detection of cocaine or cocaine metabolites; and the detection of opiates correlates well with urine based testing. The detection of cannabinoids in oral fluids compared to the detection of cannabinoids in urine did not correlate as well and suggests that the detection window for cannabinoids in oral fluid specimens is shorter than the detection window for cannabinoids in urine specimens, and requires additional studies.

Key Words: urine, oral fluid, drug detection

S9

Use of Lactic Acid as a Specimen Validity Indicator in Sweat

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The Federal Guidelines have proposed the addition of hair, sweat and oral fluids for the detection of illicit drugs in a workplace testing. One outstanding issue involves the use of a specimen validity indicator for sweat testing. While specimen validity markers such as creatinine and specific gravity are well established for urine specimens, and IGG has been used as a specimen validity indicator for oral fluid specimens, there is currently no marker used in sweat testing. The purpose of this study was to examine the concentration of lactic acid in worn sweat patches.

Eight volunteer were recruited to wear five PharmCheck™ Drugs of Abuse Sweat Patches. Three sweat patches were worn on the upper right arm and two were worn on the upper left arm. The skin was cleaned in accordance with the recommended application provided by PharmChem Laboratories, Inc. prior to the application of the sweat patches. One sweat patch was removed at day 1 post application, one sweat patch was removed at day 3 post application, one sweat patch was removed at day 5 post application, one sweat patch was removed at day 7 post application and one sweat patch was removed at day 14 post application. Lactic acid was determined using and ADVIA 2400 analyzer. This assay is based on the conversion of lactate to pyruvate, which also produces hydrogen peroxide, which is then used in an enzymatic reaction to generate a colored dye. The intensity of this chromogen is proportional to the concentration of lactic acid in the sweat.

The results of this analysis indicated that in general, there was an increase in the lactic acid concentrations the longer the sweat patch was worn. However, it was also observed that the lactic acid levels appeared to decrease after day 7 as evident from the results of the 14- day sweat patch. A large subject variability in lactic acid levels was also observed. This study indicates that the use of lactic acid as a specimen validity test for sweat may be a viable marker, however more research is required.

Key Words: sweat patch, lactic acid, specimen validity

S10

Increased Incidence of Alprazolam Usage Among New Mexico Drivers

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Alprazolam (Xanax) belongs to the class of psychoactive drugs called benzodiazepine anxiolytics. The common usage of Alprazolam is for depression, fear of open spaces, and premenstrual syndrome. In the past 2 years the State of New Mexico Dept of Health, Scientific Laboratory Division, Toxicology Bureau has confirmed alprazolam in an increasing number of DUI cases. 19 cases were reported in 2003, and 34 cases in 2004. Of the 53 cases analyzed, 58% were male and 42% were female. The average age of male drivers was 39 yrs (range 22-59) and the average age of female drivers was 44 yrs (range 22-59). A drug of abuse screen was performed on all drivers with alcohol levels less than 0.08 gm/lOOmL. The immunoassay screen included six classes of drugs: Benzodiazepine, Cocaine, Methamphetamine, PCP, THC, and Opiates. Quantitative analysis was performed using solid phase extraction and analyzed on a GC/MS with SIM monitoring. Blood concentrations were quantified with a mean of 0.08 mg/L and a median of 0.07 mg/L (range 0.02-0.18 mg/L). The reasons for stop/arrest included accident 57% (n=23), erratic driving 38 % (n=15), and drug facilitated sexual assault cases 5% (n=2). DRE evaluations were also obtained in 11 cases. We have seen an increase in the number of Alprazolam cases in the past two years. This is comparable to the accident rate and may be due to the impairment of psychomotor skills.

Key words: Alprazolam, DUID, Accident

S 11

Dextromethorphan Concentrations in Wisconsin Drivers

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Dextromethorphan is a synthetic analogue of codeine used in hundreds of over-the-counter medications for its antitussive effects. There have been numerous reports of dextromethorphan use as a recreational drug. Dextromethorphan is an NMDA receptor antagonist similar to PCP and ketamine. The recommended dosage in adults for antitussive use is a maximum of 120 mg per day, while recreational doses can range from 80 - 1500 mg. At low recreational doses, dextromethorphan can produce effects similar to that of marijuana. At higher doses dextromethorphan will produce disassociative effects, as well as sensory enhancement and hallucinations. Physical symptoms of abuse can include sweating, dilated pupils, elevated body temperature and blood pressure and dry mouth.

Since 1999 the Wisconsin State Laboratory of Hygiene (WSLH) has noted an increase in the incidence of dextromethorphan in samples from drivers suspected of driving under the influence of drugs (DUID). This study examines data from 108 DUID samples with reported blood dextromethorphan concentrations submitted to the WSLH from January 1999 through December 2004. The incidence of dextromethorphan increased from 8 cases in 1999 to 34 in 2003, before declining to 21 in 2004. During this same period overall drug testing in drivers increased threefold, from 589 to 1859, reflecting an increased law enforcement awareness of drugged driving.

Dextromethorphan is detected by GC/NPD analysis of a liquid-liquid basic extraction, with confirmation and quantitation by GC/MSD. When administered as an antitussive the expected therapeutic concentration is less than 5 ng/mL. Dextromethorphan concentrations in the 108 study cases ranged from <5 ng/mL to 1800 ng/mL with a mean concentration of 207 ng/mL. The majority of the positive dextromethorphan subjects were males (59%) under the age of 30, with a mean age of 29 and median of 23 years (range 16-68). The highest mean dextromethorphan concentrations observed were in males aged 16-20 years. Female subjects' age ranged from 16-62 with a mean age of 34 and median of 35 years.

One hundred four of the 108 samples included in this study were found to be positive for other drugs in addition to dextromethorphan. Thirty-four percent of the drivers tested were positive for delta-9 THC (38% positive for COOH-THC), 24% for chlorpheniramine, 18% for ephedrine/pseudoephedrine, 17% for cocaine and its metabolites and 15% for one or more benzodiazepine. The mean blood alcohol concentration for all 108 samples was 0.030 g/l00mL (range 0.0 - 0.226 g/l00mL). Sixty percent of the samples in this study did not have a measurable blood alcohol concentration.

Several cases will be reviewed in detail, including DRE evaluation information where available.

Key Words: Dextromethorphan, Impairment, Driving

S12 ERA Award Winner
Distribution of Methadone and EDDP in 100 Postmortem Cases

Jessica A. Jennings, Rebecca A. Jufer, Richard T. Gallery, and Rick Pretzler
Office of the Chief Medical Examiner, State of Delaware, 200 South Adams Street, Wilmington, Delaware,19801

Methadone, a legal synthetic opioid, has been used for the treatment of heroin and morphine addiction for over 40 years now in addition to being used as an analgesic. This drug is less addictive than its opioid counterparts, but it is still abused. Consequently, methadone accounts for a large portion of drug deaths each year. At the State of Delaware Office of the Chief Medical Examiner, 100 methadone-related deaths from September 6, 2001 through March 1, 2005 were investigated to determine the distribution of methadone and its main metabolite 2-ethylidene-l,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in these cases. Cases were divided into the following six groups based on their causes and manners of deaths: Group 1 A) drug-related deaths in which death was due to methadone only; Group IB) drug-related deaths in which death was due to methadone in combination with other drugs; Group 2) drug-related deaths in which methadone was not a contributing factor; Group 3) deaths in which methadone was an incidental finding (e.g. deaths due to trauma); Group 4) natural deaths that were aggravated by methadone; and Group 5) undetermined or pending cases.
The specimens that were analyzed in this study included vitreous humor (VH), peripheral blood (PB), heart blood, brain, gastric contents, and liver. Average concentrations of methadone in blood and vitreous humor (ng/mL) and tissue specimens (ng/g) are summarized below. Ratios of liver, brain, and vitreous to blood are also presented.

Group		Peripheral Blood	Liver	Liver/PB Ratio	Brain	Brain/PB Ratio	Vitreous Humor	VH/PB Ratio
1A	Average	950	4000	5.7	1800	2.2	260	0.27
(n=13)	n	9	8	7	8	7	10	9
	Range	330-1800	1500-5600	2.7-11	580-3500	1.5-2.9	60-840	0.10-0.70
IB	Average	640	3600	7.3	1300	2.6	160	0.30
(n = 27)	n	16	16	11	13	10	16	12
	Range	170-1500	990-9200	4.3-11	490-2900	1.4-4.2	47-470	0.10-0.65
2	Average	410	5000	36	1000	4.2	110	0.46
(n = 6)	n	3	3	3.0	3	3	3	3
	Range	82-600	2800-7900	4.7-96	700-1200	2.0-8.5	80-150	0.17-1.0
3	Average	630	5700	12	1700	2.3	180	0.26
(n = 33)	n	12	10	7	10	6	16	10
	Range	120-1400	2300-19000	5.0-35	220-5600	1.7-2.9	32-670	0.084-0.43
4	Average	400	2700	7.7	1000	3.0	120	0.30
(n = 9)	n	7	6	6	6	6	7	6
	Range	160-630	1400-5600	3.5-13	660-1500	1.8-4.1	67-200	0.11-0.43
5	Average	310	2900	8.9	1100	3.2	87	0.28
(n=12)	n	9	9	7	8	6	8	7
	Range	110-580	470-6800	4.3-19	470-1900	2.1-5.4	44-200	0.18-0.57

As the table shows, Group 1A had the maximum average concentration of methadone in peripheral blood (950 ng/mL) and brain (1800 ng/g) but not in liver. The maximum concentration in this tissue was actually seen with a case in Group 3. The concentration in this case (which involved a 34-year-old pregnant woman) was 19,000 ng/g. Liver concentrations were greater than blood concentrations in all cases. The liver-to-blood ratio for Group 1A was 5.7, and it ranged from 2.7-96 among all groups. Brain concentrations were greater than corresponding blood concentrations in all cases, and the brain-to-blood ratio ranged from 1.4—8.5 among all groups. Group 1A had the maximum mean concentration of methadone in vitreous (260 ng/mL), and the vitreous-to-blood ratio was <1 in all cases. Ratios of parent drug to metabolite were also evaluated in this study. The mean methadone-to-EDDP ratios in peripheral blood were not significantly different between groups. The highest mean methadone-to-EDDP ratio was observed for Group 1A (average = 11; n = 8; range = 5.0-24). The extent of redistribution was assessed by calculating heart blood-to-peripheral blood ratios. The average ratio for all six groups was 1.3 (n = 47; range = 0.54-9.5). These findings re-emphasize the importance of obtaining additional information when evaluating the role of methadone in death. Methadone toxicity is difficult to interpret due to overlapping therapeutic and lethal concentrations. Several factors contribute to this overlap including tolerance, individual variability in metabolism and postmortem redistribution. In addition, our results suggest that additional specimens such as brain or vitreous humor may be useful for evaluation of methadone-related deaths, although these cases still remain challenging when little information is known (e.g. dosing history).

S12 ERA Award Winner
Distribution of Methadone and EDDP in 100 Postmortem Cases

Jessica A. Jennings, Rebecca A. Jufer, Richard T. Callery, and Rick Pretzler
Office of the Chief Medical Examiner, State of Delaware, 200 South Adams Street, Wilmington, Delaware, 19801

Methadone, a legal synthetic opioid, has been used for the treatment of heroin and morphine addiction for over 40 years now in addition to being used as an analgesic. This drug is less addictive than its opioid counterparts, but it is still abused. Consequently, methadone accounts for a large portion of drug deaths each year. At the State of Delaware Office of the Chief Medical Examiner, 100 methadone-related deaths from September 6, 2001 through March 1, 2005 were investigated to determine the distribution of methadone and its main metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in these cases. Cases were divided into the following six groups based on their causes and manners of deaths: Group 1A) drug-related deaths in which death was due to methadone only; Group 1B) drug-related deaths in which death was due to methadone in combination with other drugs; Group 2) drug-related deaths in which methadone was not a contributing factor; Group 3) deaths in which methadone was an incidental finding (e.g. deaths due to trauma); Group 4) natural deaths that were aggravated by methadone; and Group 5) undetermined or pending cases.

The specimens that were analyzed in this study included vitreous humor (VH), peripheral blood (PB), heart blood, brain, gastric contents, and liver. Average concentrations of methadone in blood and vitreous humor (ng/mL) and tissue specimens (ng/g) are summarized below. Ratios of liver, brain, and vitreous to blood are also presented.

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	Range	330-1800	1500-5600	2.7-11	580-3500	1.5-2.9	60-840	0.10-0.70
1B	Average	640	3600	7.3	1300	2.6	160	0.30
(n = 27)	n	16	16	11	13	10	16	12
	Range	170-1500	990-9200	4.3-11	490-2900	1.4-4.2	47-470	0.10-0.65
2	Average	410	5000	36	1000	4.2	110	0.46
(n = 6)	n	3	3	3.0	3	3	3	3
	Range	82-600	2800-7900	4.7-96	700-1200	2.0-8.5	80-150	0.17-1.0
3	Average	630	5700	12	1700	2.3	180	0.26
(n = 33)	n	12	10	7	10	6	16	10
	Range	120-1400	2300-19000	5.0-35	220-5600	1.7-2.9	32-670	0.084-0.43
4	Average	400	2700	7.7	1000	3.0	120	0.30
(n = 9)	n	7	6	6	6	6	7	6
	Range	160-630	1400-5600	3.5-13	660-1500	1.8-4.1	67-200	0.11-0.43
5	Average	310	2900	8.9	1100	3.2	87	0.28
(n = 12)	n	9	9	7	8	6	8	7
	Range	110-580	470-6800	4.3-19	470-1900	2.1-5.4	44-200	0.18-0.57

As the table shows, Group 1A had the maximum average concentration of methadone in peripheral blood (950 ng/mL) and brain (1800 ng/g) but not in liver. The maximum concentration in this tissue was actually seen with a case in Group 3. The concentration in this case (which involved a 34-year-old pregnant woman) was 19,000 ng/g. Liver concentrations were greater than blood concentrations in all cases. The liver-to-blood ratio for Group 1A was 5.7, and it ranged from 2.7–96 among all groups. Brain concentrations were greater than corresponding blood concentrations in all cases, and the brain-to-blood ratio ranged from 1.4–8.5 among all groups. Group 1A had the maximum mean concentration of methadone in vitreous (260 ng/mL), and the vitreous-to-blood ratio was ≤1 in all cases.

Ratios of parent drug to metabolite were also evaluated in this study. The mean methadone-to-EDDP ratios in peripheral blood were not significantly different between groups. The highest mean methadone-to-EDDP ratio was observed for Group 1A (average = 11; n = 8; range = 5.0-24). The extent of redistribution was assessed by calculating heart blood-to-peripheral blood ratios. The average ratio for all six groups was 1.3 (n = 47; range = 0.54-9.5).

These findings re-emphasize the importance of obtaining additional information when evaluating the role of methadone in death. Methadone toxicity is difficult to interpret due to overlapping therapeutic and lethal concentrations. Several factors contribute to this overlap including tolerance, individual variability in metabolism and postmortem redistribution. In addition, our results suggest that additional specimens such as brain or vitreous humor may be useful for evaluation of methadone-related deaths, although these cases still remain challenging when little information is known (e.g. dosing history).

S13 ERA Award Winner

An Improved Method To Determine Ethyl Glucuronide In Urine Using Reversed-Phase HPLC AND Pulsed Electrochemical Detection

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There is a need for a method to distinguish between ethanol levels detected in biological matrices due to alcohol consumption versus ethanol production after death as a result of decomposition. Ethyl glucuronide (EtG) is a non-volatile, water-soluble, direct metabolite of ethanol that can serve as a biomarker of alcohol consumption for up to 80 hours after complete alcohol elimination from the body. It is an intermediate marker of alcohol consumption, bridging the gap between long-term (CDT, MCV & GOT) and very short-term (ethanol & HTOL) biomarkers. Several important clinical and forensic applications for the binary test for EtG in the urine exist. Clinical applications for this biological marker would include monitoring patients in treatment for alcohol abuse. Furthermore, by detecting and monitoring EtG in pregnant women fetal alcohol syndrome could be avoided. EtG exhibits very high storage stability, of key importance as forensic samples are often stored for extended periods of time, sometimes months or years until the case goes to court. Previous methods to detect EtG have been gas chromatography (GC) coupled with mass spectrometry (MS), and liquid chromatography (LC) coupled with MS. GC/MS is available at almost all forensic facilities at a moderate cost, requiring derivatization prior to analysis. LC/MS is advantageous because it doesn't require derivatization nonetheless it is an expensive technique. For widespread use of EtG as a marker, simpler and less expensive methods are necessary.

Previously, a method was developed for the detection of EtG in postmortem urine samples using reversed-phase liquid chromatography with pulsed electrochemical detection (PED). The current method is an improvement of the previous method in several key ways. The mobile phase previously consisted of 1% acetic acid/water and acetonitrile (98:2) and now t-butanol is serving as the organic modifier because acetonitrile was found to suppress the signal of the glucuronide. The result of this replacement is a 5-fold increase in signal of the analyte of interest. This amperometric detection technique applies alternated positive and negative potential pulses at a noble metal electrode. The analyte is oxidized followed by oxidative and reductive cleaning steps. The analyte concentration is determined by measuring the electric current resulting from the molecule gaining or losing electrons. Solid-phase extraction recoveries for EtG from urine have been improved to 70% from the previously reported 50% using aminopropyl columns. Due to the fact that the previous internal standard peak methyl glucuronide (MetG) lay within the interference of the matrix, propyl glucuronide which is baseline resolved and eluting out of the interferences has replaced MetG as the internal standard. The LOQ and LOD for EtG were improved to 0.1 and 0.03 ug/mL from previous values of 0.4 and 0.1 ug/mL respectively. Blind studies in urine showed no significant difference between the results and the true value determined at the 95% confidence level, for all samples. This method is specific, reproducible and sensitive. Reversed-phase chromatography enabled a simple separation of the analyte without requiring the ion-pairing reagents typically associated with ion chromatography. PED is a direct (no derivatization) and affordable detection method. This method is a potential tool to clinical and forensic toxicologists for determining alcohol consumption in live and deceased individuals.

Key Words: Alcohol, Biomarker, Direct and affordable analysis

S14

Rapid Simultaneous Determination of Amphetamine (AMP), Methamphetamine (MAMP), 3,4-Methylenedioxyamphetamine (MDA), 3,4-Methylenedioxymethamphetamine (MDMA) and 3,4-Methylenedioxyethylamphetamine (MDEA) in Urine by Fast Gas Chromatography-Mass Spectrometry

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The use of fast gas chromatography - mass spectrometry (FGC-MS) was investigated to improve the efficiency of analysis of urine specimens that have previously screened presumptively-positive for amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and/or 3,4 methylenedioxyethylamphetamine (MDEA) by immunoassay testing. Specimens were pretreated with basic sodium periodate, extracted using a positive-pressure manifold / cation-exchange solid-phase cartridge methodology, and derivatized using 4-carbethoxyhexafluorobutyryl chloride (4-CB). The analytical method was compared to traditional gas chromatography-mass spectrometry (GC-MS) analysis and evaluated with respect to assay linearity, sensitivity, precision, accuracy, and reproducibility.

The limit of detection (LOD) was 62.5 ng/mL for MDA and 31.25 ng/mL for AMP, MAMP, MDMA, and MDEA. All of the target analytes were linear to 12000 ng/mL with the exception of MAMP which was linear to 10000 ng/mL. The within-run precision of a multi-constituent 500 ng/mL control (n=l 5) ranged from 522.6-575.9 ng/mL with a coefficient of variation of less than 3.8%. Authentic human urine specimens (n =187) previously determined to contain the target analytes were re-extracted and analyzed by both FGC-MS and currently-utilized GC-MS methods. No significant differences in specimen concentration were observed between these analytical methods. No interferences were seen when the performance of the FGC-MS method was challenged with ephedrine, pseudoephedrine, phenylpropanolamine, and phentermine. When compared to traditional GC-MS analysis, FGC-MS analysis provided a dramatic reduction in retention time for amphetamine (4.12 minutes vice 1.8 minutes). By example, the FGC-MS method would reduce overall run time for a batch of 56 specimens from 12.0 hours to 7.25 hours. This reduction in analysis time makes FGC-MS an attractive alternative to traditional GC-MS by allowing a laboratory greater flexibility in the purchase and use of capital equipment and in the assignment of laboratory personnel, all resulting in greater overall efficiency by decreasing reporting times for AMP, MAMP, and designer amphetamine positive specimens.

Key Words: Fast GC-MS, Amphetamines, Designer drugs.

S15

Rapid Quantification of Urinary ll-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid Using Fast Gas Chromatography - Mass Spectrometry

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Human urine specimens that were determined to be presumptively-positive for metabolites of Δ 9-tetrahydrocannabinol by immunoassay screening were assayed using a novel fast gas chromatography / mass spectrometry (FGC-MS) analytical method to determine whether this method would improve the efficiency of specimen processing without diminishing the reliability of metabolite identification and quantification. Urine specimens were spiked with deuterated internal standard, subjected to solid-phase extraction, and derivatized using tetramethylammonium hydroxide and iodomethane. The methyl ester / methyl ether derivatives were identified and quantified using both a traditional GC-MS method and the newly-developed FGC-MS method.

The FGC-MS method was demonstrated to be linear between 3.8 and 1500 ng/mL 11-nor- Δ 9-tetrahydrocannabinol-9-carboxylic acid (11 -nor- Δ 9-THC-COOH). The intra-run precision of fifteen replicates of a 15 ng/mL control and the inter-run precision of 161 sets of 7,15, and 60 ng/mL controls were acceptable (coefficients of variation < 5.5%). The FGC-MS method was demonstrated to be specific for identifying 1 l-nor- Δ 9-THC-COOH and none of 43 tested substances interfered with identification and quantification of 1 l-nor- Δ 9-THC-COOH. Excellent data concordance ($R^2 > 0.993$) was found for the two specimen sets assayed using both methods. The FGC-MS method, when compared with a traditional GC-MS method, reduces total assay time by approximately 40% with no diminution in data quality.

Key Words: Fast GC, GC-MS analysis, THC.

S16

Detection of Flunixin in Equine Serum and Urine

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Flunixin (FL) is a synthetic non-steroidal anti-inflammatory drug (NSAID) and cyclooxygenase inhibitor commonly manufactured as FL meglumine (Banamine®). It is FDA-approved for veterinary medicine to treat inflammation, pyrexia, and colic in horses and cattle. The drug may be given IV, IM, and orally. FL is four times as potent as phenylbutazone and twice as potent as ketoprofen. FL is metabolized and eliminated in urine but a number of reports showed that it remains in biological specimens collected from racehorses several hours after administration of a single dose of the drug. In February 2005 the Illinois Racing Board issued new rules for medication establishing threshold levels for a few therapeutic compounds and environmental contaminants in biological specimens. The threshold level adopted for FL in serum was 20 ng/mL.

The aim of this study was to develop and validate a GC-MS method for quantitation of FL in equine serum samples. The second goal was to establish a withdrawal time in horses if FL is used therapeutically (the concentration of the drug is below 20 ng/mL in post race serum). Blood and urine samples were collected from the research mare according to the following schedule: before FL administration, at 15 and 30 minutes, and at *1*,*2*, 4, 6, 24 and 48 hours after administration of a single 500 mg IV dose of Banamine ® . After the withdrawal time was established, six horses were injected with the same dose of FL and blood samples were collected 24 and 48 hours later.

The analysis of serum (1 mL) and urine (2 mL) samples was performed using solvent extraction followed by EI-GC-MS analysis after derivatization with BSTFA at 65°C for 30 minutes. Hexobarbital was used as an internal standard for serum quantitation. The following ions were monitored: for FL *m/z* 353 (used for quantitation), and, 263, 368, and for hexobarbital *m/z* 293. The standard curve for FL in serum ranged from 10 - 100 ng/mL. In order to validate the method, two levels of controls prepared in naive horse serum were analyzed on different days (30 and 75 ng/mL).

The concentrations of FL in serum after administration of a single dose of the drug ranged from 10 µg/mL at the 15-minute time point to less than 10 ng/mL at the 48-hour time point. In urine, the estimated concentration range was from 160 µg/mL to 130 ng/mL. In three out of six horses the concentration of FL was above 20 ng/mL 24 hours after drug administration. All concentrations of FL in serum collected 48 hours after drug administration were below the threshold level. It is therefore recommended that administration of a single dose of FL is 48 hours prior to post-time. Since February, the laboratory received 25 urine samples tested positive for FL. The concentration of FL in corresponding serum samples ranged from 0 to 20 ng/mL.

Key Words: race horses, flunixin, withdrawal time, GC-MS

S17

Amitriptyline Related Fatalities in Ontario

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There is limited literature that examines amitriptyline-related fatalities. This study examines 108 cases in the province of Ontario between 2000 and 2003 in which amitriptyline and/or nortriptyline were classified as the cause of death, or where these drugs were considered to be incidental findings. In 12 cases where amitriptyline and its pharmacologically active metabolite nortriptyline were the sole drugs detected, the respective concentrations ranged from 0.6 to 27 mg/L and 0.1 to 4.1 mg/L, and were attributed as the cause of death. Other cases examined (n=36) included those involving multiple drugs where amitriptyline and nortriptyline were thought to have caused or contributed to death, but also included other drugs detected at therapeutic or lower levels. Amitriptyline and nortriptyline concentrations in these cases ranged from 0.2 to 50 mg/L and 0.05 to 12 mg/L, respectively. By comparison, in instances where death was not attributed to drug overdose and where amitriptyline and/or nortriptyline were considered to be incidental findings either alone or in combination with other drugs at therapeutic or lower concentrations (n=60), blood amitriptyline and/or nortriptyline concentrations ranged from not detected to 0.6 mg/L and not detected to 1.0 mg/L, respectively. The present study indicates a range of post-mortem re-distribution ratios (calculated from heart and peripheral blood samples; n=33) for amitriptyline and nortriptyline of between 0.26 and 11 (average 2.5), and between 0.71 and 14 (average 2.7), respectively. Additionally, other possible measures to assist with the forensic interpretation of postmortem amitriptyline and nortriptyline concentrations are discussed, including the examination of the ratio of parent drug to metabolite and the toxicity of combined concentrations of amitriptyline and nortriptyline.

Key Words: Tricyclics, Toxicity, Fatality

S18

A Fatal Therapeutic Misadventure: Mismanagement of Pain With Excessive Over Prescribing of Opiate Drugs

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Today in modern medical practice, the use of morphine and its semi-synthetic derivatives remains the cornerstone of the treatment of unrelieved pain whether due to trauma, cancer or non-cancerous conditions. It is generally accepted that the use of these drugs in pain management does not contribute to the increase in the adverse health consequences of opiate analgesic abuse. However, this conventional wisdom has lead to some physicians falling to exercise due care and proper prescribing of opiate drugs.

Objective: To alert the toxicology community to a fatal case of therapeutic misadventure due to excessive and indiscriminate prescribing of opiate drugs.

Case History: A 37 year-old female suffering from degenerative joint disease had undergone surgery for repair of a perforated left temporomandibular joint meniscus. For two months post operation, her chronic unrelieved pain was unsuccessfully treated with physical therapy, non-steroidal anti-inflammatory agents and moderate doses of oxycodone, 15mg/day. She then visited a self-proclaimed expert in pain management, Dr. X. Within four days, she had received the following drugs by prescription from Dr. X and a pharmacy specializing in Dr. X's practice: 250 Hydromorphone 4mg, 2-4 q4-6h; 150 immediate release morphine 15mg, 4-8 q4-6h; 600 oxycodone 5mg, 1-2 q6-8h; 100 sustained release oxycodone 40 mg, 1-2 q6-8h; 50 methadone 5mg, 1-2 q6-8h; 350 carisoprodol 300mg, 2-4 q2-4h; and 250 hydroxyzine, 2-3 q4-6h. Following the visit to Dr. X, the patient began the prescribed therapy, became excessively sedated with mental confusion, made telephone calls to Dr. X's office for consultation and was found dead five days after starting the new drug regimen. This case was one of 22 cases presented in hearings to suspend Dr. X's license to practice medicine and revoke the pharmacy's license.

Autopsy Findings: Unremarkable with the exception of marked blood tinged pulmonary edema, slight fatty change in the liver and evidence of her recent surgery.

Toxicology Findings: The following drugs were identified and quantified in blood by a combination of gas chromatography/ nitrogen phosphorus detection, gas chromatography/mass spectrometry or radioimmunoassay: Oxycodone, 1,100 ng/mL; morphine, total 990 ng/mL, free 100 ng/mL; methadone, 300 ng/mL; hydroxyzine, 900 ng/mL; carisoprodol, 13 mg/L; meprobamate, 11 mg/L and acetaminophen, 11 mg/L. Ethanol and other volatiles were not detected.

Conclusion: Cause of the patients' death was determined to be from "narcotic overdose". This case illustrates the dangers of excessive and indiscriminate prescribing of opiate drugs.

Key Words: Opiates, Pain Management, Fatal Overdose

S19

Evaluation of Occupational Exposure to Methamphetamine in Workers Preparing Training Aids for Drug Detection Dogs

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As a part of ongoing testing of personnel preparing training aids for drug detection dogs at Navy Criminal Investigative Services, personnel handling methamphetamine were tested. This provided a model of the potential for unwitting or environmental exposure to contribute to methamphetamine concentrations in urine. Urine samples were collected, predominantly the morning after handling drug, from multiple individuals from 2002-2004 both before and the day after the individuals had handled methamphetamine in gram quantities in the production of training aids. Personnel wore personal protective equipment including gloves, dust mask and lab coat. A total of 101 samples were analyzed for the presence of methamphetamine and amphetamine. All samples were analyzed by Gas Chromatography-Mass Spectrometry after solid phase extraction and derivatization. Methamphetamine was not detected in urine samples prior to handling drug. Samples collected after handling drug yielded a mean methamphetamine concentration of 18 ng/mL with a max of 262 and a minimum of 1.6 ng/mL when detected. Thirty-seven of the 52 post drug handling samples had detectable methamphetamine in them. Only one sample had a concentration greater than 50 ng/mL. None of the samples had detectable amphetamine present. From this limited study it was evident that handling of methamphetamine under these conditions led to minimal exposure and small but detectable concentrations of methamphetamine in urine.

Key Words: Methamphetamine, urine testing, environmental exposure

S20

Validation of the Immunalysis® Microplate ELISA for Detection of Buprenorphine and its Metabolite Norbuprenorphine in Urine

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A new enzyme-linked immunosorbent assay (ELISA) kit produced by the Immunalysis Corporation has become commercially available for the purpose of screening urine samples for buprenorphine. In this study, the kit was validated and subsequently used to screen urine samples collected from heroin addicts on the Subutex® treatment programme. Some prison, road traffic and post-mortem samples were also studied. All of the samples screened were confirmed using liquid chromatography - mass spectrometry.

At a low concentration of norbuprenorphine (1 ng/mL), the immunoassay demonstrated a cross-reactivity of 78 %. A higher cross-reactivity of 116 % was observed at a higher concentration of norbuprenorphine (10 ng/mL). Dihydrocodeine, codeine, tramadol, morphine, propoxyphene, methadone and EDDP were tested at concentrations of 10 ng/mL and 10,000 ng/mL and demonstrated no cross-reactivity with the immunoassay.

The intra-assay precision was 3.8 % (n=10) at 1 ng/mL urine. The inter-assay precision was 8.6 % (n=5) at 1 ng/mL. The limit of detection (LOD) was calculated as 0.5 ng/mL urine. Of the 63 urine samples analysed to date, there were 13 true positive, 47 true negative, 3 false positive and no false negative ELISA results. Using a cut-off value of 0.5 ng/mL urine, the immunoassay demonstrated a sensitivity and specificity of 81 % and 94 % respectively.

Key Words: Buprenorphine, ELISA, LC-MS

S21

Tizanidine Distribution in a Postmortem Case

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Zanafiex® or tizanidine is a centrally acting imidazoline muscle relaxant that is structurally similar to clonidine but not to other muscle relaxants such as baclofen or the benzodiazepines. Tizanidine is an a-2-adrenergic agonist used to treat symptomatic muscle spasms and chronic spasticity associated with central nervous system disorders such as multiple sclerosis. A case is presented of a 57 year-old white female who was found deceased at home by her husband. A suicide note was found at the scene. No remarkable findings were observed at autopsy. Tizanidine concentrations were determined by gas chromatography-mass spectrometry following solid-phase extraction and formation of the di-TMS derivative. Selected-ion-monitoring was performed for *m/z* 397, 362, and 214 for tizanidine, and *m/z* 433 and 398 for the internal standard (7-aminoclonazepam-d4). Comprehensive toxicological analysis of the heart blood identified ethanol (0.16g/dL), diazepam (l.lmg/L) and tizanidine (2.3mg/L). Blood concentrations of tizanidine following therapeutic use do not exceed 0.025mg/L. The medical examiner ruled that the cause of death was combined ethanol and multiple drug intoxication and the manner of death was suicide.

Table I. Distribution of tizanidine in the presented case.

Specimen	Tizanidine Concentration
Heart Blood (mg/L)	2.34
Urine (mg/L)	0.055
Liver (mg/kg)	9.19
Bile (mg/L)	3.37
Gastric (mg)	10

Key Words: Tizanidine, overdose, postmortem

S22

Spectrophotometric Detection of Iodide in Urine after Oxidation to Iodine

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Tests for oxidizing adulterants in urine are a continued challenge to the drug-testing program. Iodine was found to destroy morphine and 6-acetylmorphine almost immediately. The effects were less evident on ll-nor-delta-9-THC-9-carboxylic acid. When the urine solution was tested for iodine by a chromogenic substrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), no iodine was detected. Masking drug and adulterant simultaneously made iodine a preferred oxidizing adulterant for drug abusers. In this study, the reduced iodide was oxidized by sodium nitrite to iodine. The excess nitrite was decomposed by sulfamic acid and the iodine was detected by ABTS. Linearity was 12.7 to 635 mg/L (0.1 to 5 mmol/L, y = 0.9966x + 0.0016, R^2 - 1.0000). Precisions (CV%) were within + 4.1% and quantitative accuracies were within 97% of expected values (N=5). Chromate, iodate, periodate and persulfate interfered with the method. To alleviate the problem, the positive specimens were tested again by an iodine-specific method. After oxidation, the samples were treated with sodium azide and ammonium thiocyanate. In presence of thiocyanate, the azide reduced iodine to iodide almost immediately and the solutions showed negative response to ABTS. The results were compared with that of a control group tested without thiocyanate. When iodine was present, the ratios of thiocyanate and control were less than 6% whereas for interfering agents the ratios were >90%. The method showed urine background less than 1.27 mg/L (<0.01 mmol/L). It indicated that a response more than ten times of the background could be considered as iodine contamination or adulteration of urine specimens.

Key Words: Urine drug testing, Adulteration, Iodine effects and detection

S23

Postmortem Donepezil and Memantine Concentrations

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Objective:

The objective of this presentation is to provide postmortem toxicological data of two drugs utilized in the treatment of Alzheimer's disease namely, memantine (Namenda®) and donepezil (Aricept®).

Introduction:

Donepezil acts as an acetylcholinesterase inhibitor for the treatment of mild to moderate Alzheimer's disease. Donepezil's limited success is due to its inability to stop the process of neurodegeneration. As a result, new drugs, such as memantine, that focus on preserving the surviving neurons by preventing degeneration, are being employed, often in conjunction with donepezil. Steady state plasma concentrations in a therapeutic setting range from 0.07-0.15 mg/L and 0.017-0.031 mg/L for memantine and donepezil, respectively. Both drugs have high volumes of distribution (9-15 L/kg) and long half lives (50-100 hours). A case report involving the suspicious death of a 62 year old Caucasian male, who was prescribed both memantine and donepezil, is presented. Examination at autopsy showed no anatomical cause of death. In addition, results from an antemortem sample from a volunteer prescribed 5 mg/day of donepezil are presented for comparison. Method:

Memantine was extracted from the samples by a standard liquid-liquid extraction for basic drugs utilizing D-butyl chloride:ether and ammonium hydroxide. Donepezil was isolated by standard solid-phase extraction for basic drugs. Both sets of extracts were analyzed by gas chromatography/mass spectroscopy operating in selected ion monitoring mode using alphaprodine and flurazepam as internal standards for memantine and donepezil, respectively. Results:

The toxicological findings are shown in Table 1. The method of standard addition was employed to rule out any matrix effect in the liver. The distribution of both drugs are similar with the highest concentration being present in the liver and the lowest in the vitreous humor. The heart/peripheral blood ratio for memantine and donepezil is 2.7 and 2.0 respectively, which suggests postmortem redistribution. The results of donepezil in this case report are consistent to those reported by the Los Angeles Coroner's Office and two other NCOCME cases, which have causes of death unrelated to toxicity.

Table 1: Drug concentrations in presented case report and volunteer.

Matrix	Memantine	Donepezil
Blood (aorta) (mg/L)	1.8	0.71
Blood (iliac) (mg/L)	0.65	0.35
Urine (mg/L)	6.2	1.9
Gastric (mg/kg)	6.2	2.1
Vitreous Humor (mg/L)	0.39	0.26
Liver (mg/kg)	6.1	5.1
Blood (antemortem) (mg/L)	NA	0.03

Conclusion:

Upon reviewing the data, it was concluded that the results are not indicative of a drug overdose. The concentrations obtained for donepezil compare to the incidental findings reported by the Los Angeles Coroner's Office. There is currently no information regarding postmortem memantine concentrations. The laboratory will continue to compile postmortem donepezil and memantine data in an effort to acquire more data that will aid in the interpretation of future cases.

Key Words: Memantine, Donepezil, Postmortem

S24

Mivacurium and Metabolites in Biological Samples by LC Fluorescence and LC Tandem Mass Spectrometry

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Mivacurium chloride (Mivacron®) is a short-acting, nondepolarizing skeletal muscle relaxant with a quaternary amine structure. It is administered to surgical patients under general anesthesia in order to stop involuntary movement of their internal organs. When administered to an individual not on a ventilator, it leaves the victim unable to breath, and can cause death. A method for screening and confirming mivacurium and its alcohol and ester metabolites in biological fluids is presented here.

Following addition of tubocurarine as the internal standard, and protein precipitation with acetonitrile, samples are applied to pre-conditioned solid phase extraction columns (Maxi-Clean IC-RP), and the eluent is collected. The eluent is taken to dryness, and reconstituted in 0.1 mL liquid chromatography mobile phase. Analysis of the extracts is by liquid chromatography with fluorescence detection (LC/FLU) and liquid chromatography with tandem mass spectral detection (LC/MS/MS). The column used for the FLU assay is a 15 cm x 5 *i* x 4.6 mm Lichrospher 60 RP-Select from Alltech. The mobile phase is acetonitrile and 0.005 M octanesulfonic acid in a gradient. Under the conditions employed, all analytes are baseline separated and elute within 12 minutes. The isomers of mivacurium and its ester metabolite are nearly baseline resolved with this method. The column used for the MS/MS analysis is the same, except that it has a 2.1 mm i.d. The mobile phase for this analysis is 40% acetonitrile/60% water/0.025% methanesufonic acid, at a flow rate of 0.3 mL/min. Analytes are eluted under these conditions within 10 minutes. Precursor ions for the MS/MS experiment were 609 m/z (for the internal standard), 514 m/z (for mivacurium), 600 m/z (for the mivacurium ester metabolites) and 446 m/z (for the mivacurium alcohol metabolite).

Lower limits of detection for mivacurium, its alcohol metabolite, and its ester metabolite are as follows: 50, 10, and 10 ng/mL. The lower limit of quantitation for each analyte is 100 ng/mL.

This method was used to analyze several biological specimens from individuals suspected to have been exposed to mivacurium. Several of these specimens were clinical samples, while most were postmortem samples from exhumed bodies. The results of this testing, as well as more details of the method itself, will be presented.

Key Words: Quaternary Amine, Mivacurium, LC/MS/MS

S25

Investigation of Fatalities Due to Acute Gasoline Poisoning

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Abstract

This paper presents a simple, rapid, reliable, and validated method suited for forensic examination of gasoline in biological samples. The proposed methodology has been applied to the investigation of four fatal cases due to gasoline poisoning that occurred in Spain in years 2003 and 2004. Case histories, and pathological and toxicological findings are described here to illustrate the danger of gasoline exposure under several circumstances. The gasoline tissular distribution, its quantitative toxicological significance, and the possible mechanisms leading to death are also discussed. The toxicological screening and quantitation of gasoline was performed by means of gas chromatography with flame ionization detector (GC-FID) and confirmation was performed using gas chromatography-mass spectrometry (GC-MS) total ion chromatogram (TIC) mode. m,p-Xylene peak was selected to estimate gasoline in blood and other tissues. Gasoline analytical methodology was validated at five concentration levels from 1 to 100 mg/L. The method provided extraction recoveries between 77.6% and 98.3%. The limit of detection (LOD) was 0.3 mg/L and the limit of quantitation (LOQ) was 1.0 mg/L. The linearity of the blood calibration curves was excellent with r2 values of > 0.997. Intraday and interday precisions were acceptable with a coefficient of variation < 5.4% in all cases. Cases 1 and 2 consist of the accidental inhalation of gasoline vapor inside a small enclosed space. Case 3 is a death by recreational gasoline inhalation in a male adolescent. Heart blood concentrations were respectively 28.4, 18.0, and 38.3 mg/L; liver concentrations were 41.4, 52.9, and 124.2 mg/kg, and lung concentrations were 5.6, 8.4 and 39.3 mg/kg, respectively. Case 4 was an accidental death of a woman with senile dementia due to gasoline ingestion. Peripheral blood concentration was 122.4 mg/L, the highest in our experience. Since pathological findings were consistent with other reports of gasoline intoxication and constituents of gasoline were found in the body, cause of death was attributed to acute gasoline intoxication. As a rule, this kind of poisoning offers little difficulty in diagnosis because there is a history of exposure, and the odor usually clings to the clothes, skin or gastric contents. However, anatomic autopsy findings will be nonspecific and therefore toxicological analysis is necessary. There is a paucity of recent references regarding analytical and toxicological data, this article provides with evidence about toxic concentrations and is a useful adjunct to the postmortem toxicological interpretation of fatalities if the decedent has been involved in gasoline use. Besides, with complex compounds such as petroleum distillates, the development of standardized methodology, in order to be able to compare data from different authors in different laboratories, is urgently needed.

KeyWords: Gasoline, Investigation, Fatalities

S26
Methadone Strikes Again?

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While certifiying toxicology results at the Office of the Chief Medical Examiner in North Carolina, it appeared that there were an extraordinary number of deaths involving methadone in which the accompanying information did not suggest it should be present. A study was performed whereby cases that were certified in a 2-month period between February and April, 2005 were examined to see if this was a true phenomenon or if it was just anecdotal. In doing so, it was also seen if the same was true of any other drugs and if so, what were they.

A total of 164 toxicology-related cases were reviewed in the study and they were divided into the following groups:

Correctly identified agents as possibly being involved in death (n=75+32= 107)
Correctly identified methadone (n=32)
Correctly identified other agent(s) (n=75)
(What are other agents?)
Incorrectly identified agents as possibly being involved in death (n=32+7+18=57)
Methadone found unexpectedly (n=32)
(What agent(s) were expected?)
Methadone expected but not found (n=7)
(What agent(s) were found?)
Other agent(s) found unexpectedly (n=l8)
(What were they?)

The medical examiner's (ME) preliminary investigation correctly identified the agent(s) that may have been significant in determining the cause and manner of death in 107 (65%) of the cases reviewed in this study. In 32 (30%) of these cases, methadone was named as one of the agents while 75 (70%) of the cases involved agents other than methadone. Of the cases where agents other than methadone were correctly identified as possibly being involved in the death, cocaine was the single most prevalent drug. Opioids were clearly the most prevalent group, specifically; oxycodone>fentanyl>morphine>hydrocodone>propoxyphene>heroin>codeine. Following opioids, were the antidepressants; amitriptyline>sertraline>bupropion=fluoxetine= venlafaxine>citalopram=trazadone, and the benzodiazepines; diazepam>alprazolam>lorazepam>clonazepam=temazepam.

The remainder of the cases 57 (35%) reviewed in this study contained information from the preliminary ME investigation that either had no direct effect on the cause and manner of death or that omitted a significant agent that could have been involved. Of these, 32 (56%) were positive for methadone when it was not expected to be present, 7 (12%) were negative for methadone when it was expected, and 18 (32%) contained agents, other than methadone, that were not mentioned in the investigation. Of the 7 cases where methadone was expected but not found, morphine, heroin, oxycodone, propoxyphene, and fentanyl were detected, with equal frequency, in addition to paroxetine, sertraline, cocaine, methamphetamine, MDMA, and quetiapine. Ethanol was the most frequently detected agent followed by amitrityline and diazepam. The 18 cases in which other agents were found but not expected contained cocaine>fentanyl>heroin=morphine= propoxyphene=hydrocodone>oxycodone=amitriptyline=fluoxetine=citalopram=metaxalone > paroxetine=venlafaxine=alprazolam=phenytoin=lamotrigine=meperidine=tramadol.

Sixty-five percent of the time, the information that accompanied the biological specimens to the lab was accurate Thirty-five percent of the time, the history or circumstances didn't correlate with the toxicological findings. In 56% of those cases, methadone was present. Clearly, the observation that methadone is frequently present when it is not expected is not just anecdotal but a real phenomenon. It was discovered that cocaine is second to methadone at being present when not expected.

Key Words: Methadone, Medical Examiner, History

S27

Use Of Tetrahydroziline (Visine®) For Chemical Submission And Sexual Assault In Children

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Objective: This is a report of the use of a commonly available over-the-counter drug to induce an obtunded compliant victim with no memory of the period during the sexual assault. It provides the results from a police investigation into crimes against children as well as the investigation of the method used by the perpetrator of the crimes to repeatedly allow assault while inhibiting resistance or any memory of the time under sedation. It will also discuss toxic mechanism of tetrahydrozoline.

Case report: In 2003 police investigation located pictorial evidence on the Internet of adults having sex with children. Further investigation located these children in the United States and they were removed from the home. An adult male relative with sole legal custody had primary care of the children and was a suspect in the investigation. The 4 female children were 2 years old through 8 years old at the time the abuses occurred. After being removed from the home the children were interviewed and entered into counseling. However, during all interviews and counseling sessions over the subsequent year following removal from the home, the children denied having any specific memories of the sexual assaults, despite the pictorial evidence in which police could positively identify them. The children did give a history of being given a substance by their "father" when they were "bad", prior to their punishment, so that they "would not remember". The home of the male guardian was searched for drugs of abuse and those drugs known to be used in chemical submission. No drugs were located. Interviews with the wife of the suspect (not the mother of the children) indicated the suspect would give the children Visine prior to any sexual assault and the suspect would routinely carry around a bottle of Visine® with him. This history was obtained after the children had been removed from the home and so prohibited any testing of the children for tetrahydrozoline. It is unclear where the suspect learned of sedative effects of Visine®.

Visine® contains 0.05% tetrahydrozoline. Tetrahydrozoline is a central alpha-1 agonist with a similar effect to clonidine and tizanidine. Effects from unintentional and intentional ingestion may include the narcotic-like effects of sedation, coma, miosis, and respiratory depression along with the cardiovascular effects of bradycardia and hypotension. The toxic dose is poorly defined but may be as small as 2 ml of a 0.05% solution for a child. No fatalities have been reported. The effects from tetrahydrozoline may be more pronounced in children than adults. Overdoses have been reported in both children and adults, but intentional use for chemical submission has not been previously reported. One case report of a suicide attempt in an adult reports the patient learned of the effects of tetrahydrozoline from bartenders and prostitutes that had used it to subdue rowdy customers. Tetrahydrozoline is well absorbed with clinical effects evident in 15 minutes after ingestion.

Tetrahydrozoline will not be detected on routine toxicology investigation. It had been reported to produce false negative results for cannabinoids with urine immunoassay screens.

Conclusion: This case suggests that tetrahydrozoline should be added to the list of drugs suspected in cases of chemical submission and sexual assault. Along with other drugs used for this purpose, such as GHB (gama hydroxybutryrate) and ketamine, tetrahydrozoline may not be detected on routine drug screens.

Key Words: tetrahydrozoline, chemical submission, sexual assault

S28

Selenium Toxicity: Two Fatal Cases

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Objective: Selenium is a rare toxin and not routinely tested for in postmortem toxicology analysis. We report two deaths unexplained by natural causes in which continued postmortem examination, including exhumation in one case, revealed elevated selenium concentrations and a determination of the cause of death.

Results: Case one: A previously healthy 36 YO female experienced new onset vomiting, diarrhea, pallor and weakness 14 to 16 hours prior to her death. One to two hours antemortem the patient experienced chest pain, shortness of breath and syncope. Upon arrival in the ED the patient was lucid, with vomiting, abdominal pain, chest pain, hypotension and pulmonary edema. The patient's symptoms were not responsive to oxygen and dopamine. She experienced ventricular arrhythmias, shock, bradycardia and asystole. Resuscitation was unsuccessful. An autopsy performed 10 hours postmortem revealed extensive pulmonary edema and congestion. Myocardial fibers did not show necrosis and there were no changes of acute or old myocardial infarction. Heart blood for toxicology analysis showed no drugs by GC/MS. Initial cause of death was listed as undetermined. The body was given to a funeral home, embalmed and buried. Investigation revealed a significant history of marital discord and at the request of the deceased's family, the body was ordered exhumed for a second autopsy and toxicological analysis. Exhumation occurred 4 months postmortem. Analysis of liver and spleen tissues for drugs, cyanide and organophosphates was negative. Samples were analyzed in the Metals Laboratory at Mayo Clinic, using a Perkin Elmer ICP mass spectrometer. Analysis for arsenic, cadmium, lead, mercury and thallium were within normal limits. Selenium was first detected serendipitously when using the total quant algorithm. Tissue samples were sent for testing for selenium at two independent laboratories for confirmation using graphite furnace atomic absorption spectrophotometry. Kidney and liver selenium were 10.285 mcg/g and 2.65 mcg/g, respectively. The cause of death was changed to selenium toxicity.

Case two: a 36 YO male experienced new onset of abdominal pain, chest pain, nausea, vomiting and diarrhea. Upon arrival at the ED the patient complained of chest pain, abdominal pain, dizziness and vomiting and diarrhea. Over the next 14 hours the patient's condition deteriorated with hypotension unresponsive to therapy, pulmonary edema, arrhythmias and onset of acidosis. During this period, both the patient's wife of 18 years and his pregnant girlfriend separately suggested poisoning and implicated the other woman. Fifteen hours post arrival, the patient experienced shock and ventricular fibrillation deteriorating to asystole. Resuscitation efforts were unsuccessful. An autopsy was performed 24 hours post mortem and revealed extensive hemorrhagic pulmonary edema. There was no evidence of recent or old myocardial ischemia. No drags, ethanol or volatiles were found in the blood. Because of a suspicion of possible arsenic poisoning and the lack of anatomical cause of death, analysis for metals was undertaken. Analysis of postmortem blood for arsenic, bismuth, lead, mercury, barium and thallium were within normal limits. Analysis for selenium revealed 1900 mcg/L. (nl < 130mcg/L) Urine selenium was 172 mcg/L (nl <0.04 mcg/L). Cause of death was determined to be acute selenium poisoning.

Conclusion: These two cases present several important features associated with selenium toxicity, two of which are previously unreported: 1) selenium as a potential homicidal agent, 2) the toxidrome and time frame of selenium toxicity 3) selenium determination in exhumed, embalmed tissues and 4) postmortem urine selenium results. In both cases selenium was first detected serendipitously.

S29

Cyclobenzaprine (Flexeril®) Concentrations in Postmortem Cases

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Cyclobenzaprine is a tricyclic compound similar in structure to amitriptyline, differing by only one double bond. Cyclobenzaprine has been available as a centrally acting skeletal muscle relaxant since 1977. Prior to its availability as a muscle relaxant, clinical trials were conducted to evaluate the effectiveness of cyclobenzaprine as an antidepressant. During these trials, cyclobenzaprine was administered in doses up to 400 mg per day. Much lower doses of cyclobenzaprine are administered for muscle relaxant activity. For most patients, the recommended dosage is 10 mg three times per day, up to a maximum dose of 60 mg per day. Side effects reported with cyclobenzaprine use include drowsiness, dry mouth, dizziness, nausea, and less frequently, tachycardia, disorientation and hallucinations.

A mean peak plasma cyclobenzaprine concentration of 25.9 ng/mL (range 12.8 - 46.1) has been reported in subjects receiving a 10 mg dose three times per day. Cyclobenzaprine is extensively metabolized, with its major urinary metabolites being cyclobenzaprine-N-glucuronide and cis-10,11-dihydroxynortriptyline. The average plasma elimination half-life of cyclobenzaprine is 18 hours in healthy adults.

Fifty-five cyclobenzaprine positive postmortem cases were reviewed to evaluate the role of cyclobenzaprine in death investigation casework. Of these 55 cases, 37 were classified as non-drug-related deaths, 14 were drag-related deaths in which cyclobenzaprine did not play a role and four were drug-related deaths in which cyclobenzaprine contributed to the death. Cyclobenzaprine was analyzed by liquid-liquid extraction followed by GC-NPD quantitation. All positives were confirmed by GC-MS. Cyclobenzaprine concentrations (mean/median, range) for the three groups are summarized below.

Group	Heart Blood CBP (mg/L)	Subclavian Blood CBP (mg/L)	Liver CBP (mg/kg)
Non-Drug Related	0.16/0.11 (0.04-0.51) n=30	0.23/0.18 (0.07 - 0.89) n-9	0.77 / 0.56 (0.23-1.9) n=5
Drug Related - not CBP	0.20/0.17 (0.06 - 0.42) n=13	0.22/0.18 (0.07 - 0.52) n=6	2.0/2.0 n=l
Drug Related - CBP	1.2/0.68 (0.30-3.1) n=5	1.3/1.4 (1.3-1.4) n=2	31/31 (14-47) n=2

There were no cases of cyclobenzaprine overdose where cyclobenzaprine was the sole agent detected. The four cases where cyclobenzaprine was considered contributory to death also involved ethanol and/or significant concentrations of at least one other drag; three of these cases involved at least one opiate. One or more opiates were also present in 13 of the 14 drag-related deaths where cyclobenzaprine was an incidental finding. In the two groups where cyclobenzaprine was an incidental finding, the median blood cyclobenzaprine concentrations were 0.11 and 0.17 mg/L. Heart Blood / subclavian blood concentration ratios were evaluated for 14 cases. The median HB/SB ratio was 0.93 and the range was 0.4 - 2.7. These data suggest that postmortem blood cyclobenzaprine concentrations may be site dependent, but there was no clear trend for heart or subclavian blood to have a greater concentration. Liver cyclobenzaprine concentrations were in the range of 10 - 36 (n = 6) times greater than blood concentrations for the cases evaluated.

Key Words: Cyclobenzaprine, Postmortem, Blood Concentrations

S30

The Use of LC/MS/MS Technologies for Drug Screening, Confirmation and Quantification

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The forensic/toxicology analytical workflow in many respects can involve a long list of compounds that need to be screened for. This list can be as long as hundreds of compounds covering many different classes. During the screening process, potential 'hits' don't necessarily mean a positive result. The next step in the process is to verify the identity of the potential positive result and to obtain some quantitative information at the same time. LC/MS technologies have come a long way in the last 5 to 10 years and the traditional way of analyzing samples has improved significantly. Techniques to identify, confirm and quantitate analytes present in complex sample matrices have been made much easier with the development of newer hybrid instrument technologies. In this presentation, LC/MS/MS technologies will be discussed along with some automated software tools that allow for the screening, confirmation and quantitative analysis of such information.

KeyWords: LC/MS/MS, Screening, Confirmation

S31

Significance of Blood Morphine Concentrations in Non-Heroin-Related Deaths

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The majority of the morphine-related fatalities in the literature cite heroin administration. There is a paucity of literature that details morphine-related fatalities involving the use or misuse of pharmaceutical morphine formulations. Interpreting morphine concentrations in death investigations is made difficult by this lack of literature and this difficulty is compounded by the possible development of functional tolerance to morphine. In a therapeutic context, blood morphine concentrations up to 400 ng/mL have been tolerated in cancer patients receiving morphine for pain control. In contrast, concentrations of approximately 200 ng/mL and above are believed to be potentially fatal. This study was initiated to determine the significance of free-morphine concentrations below 400 ng/mL in fatalities associated with the use of morphine.

This study consisted of a retrospective examination of 40 death investigations in Northern Ontario where blood concentrations of free-morphine ranged up to 400 ng/mL. Investigations were categorized according to the cause of death (COD) as determined by the coroner as: morphine overdoses (MOD), multiple drug intoxications (MDI), unknown causes (UNK), or known anatomical cause (KAC). The mean, median and range of blood free-morphine concentrations (in ng/mL) and sample size for each COD were: MOD (131; 124; 107 to 170; n = 4), MDI (175; 118; 31 to 399; n = 22), UNK (114; 92; 68 to 177; n = 5), KAC (146; 92; 15 to 373; n = 9). No statistical differences in blood free-morphine concentrations were determined between COD groups. When cases with fatal levels of other drugs and/or positive cocaine concentrations were removed, there remained no statistical difference between the COD groups.

While the number of cases is small, this study does suggest that blood free morphine concentrations of approximately 100 ng/mL and higher should be considered as a potential contributing factor to a cause of death. For such a determination, though, other information must also be considered such as the route of morphine administration, the tolerance of the individual to morphine, the health of the individual, timeline between morphine ingestion and death, evidence from the scene and other pathological findings including pulmonary oedema and potential anatomical causes of death.

Key Words: Morphine, Fatalities, Ontario

S32

Elevated Caffeine Levels in Two Postmortem Cases and a DUI Case in New Mexico

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Caffeine is a mild central nervous system stimulant widely used in most Western societies. It is present in coffee, teas, soft drinks, cocoa, prescription and over the counter medications ranging in amounts from 25-200 mgs. The physiological effects on the body consist of myocardial and respiratory stimulation, diuresis and increased fatty acid mobilization. Caffeine is thought to act on the brain by blocking adenosine receptors. At elevated levels (typically > 300 mg dose) anxiety, insomnia, headaches and gastrointestinal irritation can occur while at toxic levels loss of appetite, flushing, weakness, tremors, tachycardia, convulsions, coma and death may result. Although deaths due to overdose are rare, our laboratory reported two fatal caffeine ingestions in 2004, as well as an elevated level detected in a truck driver involved in an accident. Case #1 involved a 40 yr old female with history of epilepsy, paranoid schizophrenia and hypertension. She was found by her husband in their residence incapacitated and suffering from blunt trauma inflicted upon her by her son. Antemortem and postmortem drug levels were: Carbamazepine 5.9 mg/L AM blood, phenytoin 2.3 mg/L AM serum, phenytoin 5.3 mg/L AM blood, caffeine 564 mg/L AM blood, caffeine 924 mg/L femoral blood, 278mg/L vitreous, 189 mg/kg liver, 648 mg/L bile and 456 mg/L heart blood. Cause of death was caffeine poisoning and manner of death was homicide. Following our results, the decedent's son was convicted of aggravated battery as opposed to murder.

hi the second case, a 25 yr old Hispanic female with a history of depression was found dead at her residence when she had failed to show up at work. Green emesis in the bathroom and an open container of Mouse D Con was found in kitchen. Green gastric contents at the autopsy lead pathologist to believe warfarin possibly could be a contributory factor in her death. Toxicological levels were as follows: Diazepam < 0.1 mg/L femoral blood, nordiazepam < 0.1 mg/L femoral blood, caffeine 240 mg/L, 702 mg/L heart blood, 126 mg/L vitreous, 4070 mg/L stomach contents, 98.9 mg/kg brain. The decedent's mother revealed her daughter had been using diet pills. The cause of death was caffeine intoxication and manner of death was undetermined.

The final caffeine case involved a truck driver who failed to negotiate a turn and ran into a stationary vehicle pinning it between his truck and a building. Witnesses observed driver speeding and swerving. Investigating officer observed signs of impairment such as jerky movements, slurred speech, nystagmus, lack of balance and dyskinesia. Driver admitted to taking caffeine pills to stay awake. Caffeine was detected at 86 mg/L.

hi summary, caffeine is one of the most widely used stimulants in the world and although considered benign in low doses, large consumption of caffeine can lead to intoxication resulting in insomnia, anxiety, gastrointestinal distress, arrhythmias, convulsions and death. Our findings illustrate the potential risks of caffeine intoxication in overdoses and driving under the influence.

Key Words: Caffeine, Driving and Postmortem

S33

Aggressive Resuscitation as a Cause of Post-Mortem Redistribution

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Postmortem Redistribution (PMR) of drugs is one of the confounding factors limiting the toxicological significance of drug concentrations in forensic investigations of deaths. This case report provides evidence for aggressive resuscitation in the Emergency Room (ER) as a possible cause for PMR and the importance of reporting resuscitation in the case history. Briefly, a 47 year old female was found "vital signs absent", with several empty bottles of medication. Aggressive resuscitation was attempted for approximately 45 minutes in the ER, during which time transient life signs were present, before being pronounced dead. As admission blood was limited in volume, screening by ELISA and a GC/MS basic drug screen was performed on a post-mortem "peripheral" blood sample.

In this case PMR was suspected when the GC/MS screen identified amitriptyline and nortriptyline in the postmortem blood, but subsequent attempts to quantify the concentrations of these drugs in the admission blood revealed they were below the limit of detection (LOD) for the GC method used. Five more drugs, including codeine, hydrocodone, oxycodone, alprazolam and acetaminophen were detected and displayed significant PMR. Comparing peripheral autopsy to admission blood concentrations produced the following ratios: Codeine - 10, Oxycodone - 9, hydrocodone - 7, amitriptyline — 6.3 (using the LOD; the actual PM concentration may have been much less), alprazolam - 4.5, acetaminophen - 1.8 (nortripyline concentrations were too low to determine a ratio).

Although high volume of distribution (Vd) is often correlated with PMR, statistical analysis revealed a poor correlation. Comparing the admission to postmortem blood ratios with published Vd's for these drugs provided correlation coefficients (R^2) ranging from 0.1017 to 0.1748 when using both low and high ends of the Vd ranges. A possible mechanism for the increase in all of these drugs would be release from the central organs, especially the liver and lungs, during resuscitation and subsequent diffusion to the "periphery" in the interval between death and the autopsy. This case provides forensic toxicologists with another aspect of the case history that may signal the possibility of PMR and the need for caution when interpreting post-mortem drug concentrations.

Key Words: Postmortem redistribution, Resuscitation, Interpretation

S34

Study of Oxycodone Occurrences at a Large City Medical Examiners' Office

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Introduction: Recent reports in the news media have indicated that use of street drugs such as Ecstasy, which are usually obtained from sources outside the United States, have declined because of increased homeland security post 9/11. It is postulated that prescription drugs such as oxycodone are being used as "safer" replacements. In addition, other reports show that because of the heightened awareness of abuse of the extended release form of oxycodone (OxyContin™) the number of legal prescriptions of the drug may be decreasing. This study from a large city MEO was undertaken to determine the frequency of oxycodone as the cause of death in 2003 and 2004 , either singly or in combination with other drugs.

Methods: Post-mortem data was obtained from a computer database (CME) for all deaths involving oxycodone for the years 2003 and 2004. Other drugs/drug classes that were detected concurrently with the oxycodone were broken down into the following groups: cocaine and metabolites, ethanol, antidepressants/antipsychotics , narcotics, PCP and benzodiazepines. All drug screen results were from fluoridated blood or urine and were confirmed by GC-MS except for benzodiazepines where indicated. No attempt was made to discriminate between the presence of any one specific product of the Schedule II oxycodone containing products available in the United States.

Results: Oxycodone: In 2003 the Toxicology laboratory at the Philadelphia Medical Examiners' Office tested specimens from 2094 cases. Eighty two (3.9.%) of these cases were positive for oxycodone. In fifty four (65.6%) of the cases positive for oxycodone, the cause of death was determined to be either drug induced or drug related. In 2004, specimens were tested from 2067 cases. One hundred two (4.9%)of these cases were positive for oxycodone. In sixty three (61.8%) of the positive oxycodone cases, the cause of death was determined to be either drug induced or drug related. Concurrence of the other drugs/drug classes in drug abuse cases followed a similar pattern in both years with benzodiazepines > narcotics > antidepressants/antipsychotics > cocaine and metabolites > ethanol > PCP. Other abused drugs: As a comparison, in 2003 cocaine, the most commonly seen drug of abuse seen at our MEO, was positive in 302 cases (14.4 % of total cases) with 171 (56.7%) of these deaths attributed to drug abuse. Similar statistics were seen in 2004: 357 positive cocaine cases (17.2% of total cases) with 209 (58.5%) of these deaths attributed to drug abuse

Conclusions: In our two-year study, no drug abuse cases (total = 107) were found where oxycodone was the sole drug present. A high prevalence of concurrent use of benzodiazepines, antidepressants/antipsychotics, narcotics and cocaine as well as occasional use of ethanol and PCP was seen. In lieu of the fact that the percentage of cases of oxycodone determined to be drug abuse cases actually increased slightly to 3.0% (63/2067) in 2004 from 2.6% (54/2094) in 2003, it is clear that oxycodone continues to be a very common drug found in overdose cases and that its role should be recognized as significant in these deaths. Consequently, special care needs to be taken to ensure that toxicology testing detects oxycodone. In many cases this requires GC/MS since cross-reactivity to oxycodone in most immunoassays for the opiate class is very low. In addition, data from our laboratory indicates that both Ecstasy and methamphetamine are infrequently seen as causes of death in Philadelphia.

Key Words: Oxycodone, Postmortem toxicology, Drugs of Abuse

S35

Amphetamine Enantiomer Distribution in Hair and Blood to Monitor Abstinence From Street Amphetamine in Adult Attention Deficit Hyperactivity Disorder (ADHD) Treatment

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The objectives of this study were to develop a method for the quantitation of the R-(-)- and S-(+)-amphetamine enantiomers in both blood and hair and to investigate the enantiomer distribution in ADHD patients, with a previous history of drug abuse, receiving amphetamine as medication. Because of the high risk of relapse into drug abuse, a strategy involving the analysis of amphetamine enantiomers in blood and hair was investigated for the assessment of compliance as well as abstinence from street amphetamine.

Four patients were monitored; one patient was treated with racemic amphetamine, and three with Metamina®. At least three blood and hair samples were obtained during the treatment. A 15 mm segment of the hair (from the scalp and out) was used for the analysis. A basic extraction of the analytes into iso-octane was used. Hair was dissolved in sodium hydroxide before extraction. Chiral derivatization was performed by reaction with S-(-)-N-(trifluoroacetyl) prolyl chloride. Quantitation of R-(-)- and S-(+)-amphetamine was performed by gas chromatography-mass spectrometry in selected ion monitoring.

LOQ was determined to 2.5 ng/hair sample and 5 ng/g of blood and coefficients of variation was lower than 10 % at three levels including the LOQ, except for S-(+)-amphetamine where the CV was 15 % at LOQ. The amphetamine enantiomer concentrations and percentage of S-(+)-amphetamine in blood and hair from the patients are shown in Table 1. The hair and blood results as well as the clinical observations of patients 1 and 2 showed no indications of noncompliance. Patient 3 and 4, on the other hand, showed different percentages of S-(+)-amphetamine in hair together with varying total concentrations. Patient 3 admitted to have taken illicit amphetamine in addition to prescribed medication during the autumn. Patient 4 was the only patient that had a positive hair sample at the inclusion, confirming amphetamine abuse. The results from the second hair sample also indicated use of illicit amphetamine in addition to the medication whereas the plasma sample is consistent with use of medication only.

We conclude that hair revealed drug abuse is not shown by concentrations or enantiomer ratios in blood. Also, we believe that the use of hair as a matrix, in contrast to plasma, prevents "pseudo-compliance" where the patient only takes his medication the days prior to sampling, while abstaining from illicit amphetamine. In addition to these control-focused benefits, the patient can also be given positive feedback on his compliance in the meeting with his clinician.

Table 1.

Patient	Dose	Sample	Blood			Hair		
	(mg/d)	(date)	R-(-) (ng/g)	S-(+) (ng/g)	S-(+) (%)	R-(-) (ng/mg)	S-(+) (ng/mg)	S-(+) (%)
1	0	2002-12-09	n.a.	n.a.	-	0	0	-
	45	2003-01-07	46.5	26.9	36.6	0.66	0.61	48.0
	50	2003-01-13	47.7	29.6	38.3	0.88	0.77	46.7
	50	2003-04-28	56.5	40.1	41.5	0.96	0.83	46.4
2	0	2003-06-23	0	0	-	0	0	-
	60	2003-11-14	26.5	177	87.0	0.32	2.36	88.1
	60	2004-04-14	15.3	114	88.2	0.28	2.08	88.1
	60	2004-11-05	29.8	194	86.7	0.21	1.70	89.0
3	0	2003-11-21	0	0	-	0	0	-
	30	2004-04-28	3.5	27.8	88.8	0.64	4.83	88.3
	20	2004-12-01	11.1	50.5	82.0	3.03	5.54	64.6
4	0	2003-11-05	0	0	-	19.5	18.8	49.1
	55	2004-03-03	6.0	37.9	86.3	52	93	64.1
	40	2004-11-15	0.6	2.4	80.0	0.66	5.04	88.4

Key Words: ADHD, amphetamine enantiomers, chiral derivatization

S36

Gas Chromatographic/Mass Spectrometric Analysis of Drugs Using Library Mass Spectral Matching and Retention Index Validation

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Drugs detected during screening by use of a gas chromatograph/mass spectrometer (GC/MS) may be quickly and reliably identified with a high degree of certainty by using a unique macro - that is, a series of programmed tasks to be performed by a computer. The macro is able to search as many libraries as available and to validate mass spectral matches with retention indices. A solution containing 33 |Lig/mL each of 27 n-alkanes with sequential carbon chain lengths, n-decane (CioH22) through n-hexatriacontane (Csef^), in n-heptane solvent was prepared for daily retention index calibration of the GC/MS. The calibration procedure is quick, simple, and is driven by the macro. A mass spectral library was created by analyzing standards of drugs and drug metabolites, artifacts, and derivatives. The library entries included retention indices. Twelve user-created libraries shared by other laboratories and purchased libraries were included in the searches. The macro was written to analyze data, post-run, on an Agilent GC/MS. This macro prints out library spectral matches validated by retention indices. It also lists additional library hits of other drugs with a poorer, but good, quality spectral correlation that may have better retention index matches. In addition, a table listing the best-quality spectral match from all available libraries for each of all other peaks not having been identified by retention index is generated for closer review by the analyst. The analytes are ultimately confirmed by comparing unknown data to that of drug standards analyzed on the same instrument under the same conditions. As a result of the use of the macro, each analyte of a drug screen is quickly and reliably identified and thoroughly searched to all available libraries loaded on the analytical instrument.

Key Words: Macro, Gas Chromatograph/Mass Spectrometer, Retention Indices

S37

Fast Gradient Elution Liquid Chromatography for Rapid Screening of Drugs of Abuse in Blood

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The objective of this presentation is to introduce a technique for screening blood samples for drugs in 2.5 minutes by liquid chromatography coupled with diode-array detection.

Gradient elution reversed-phase liquid chromatography coupled with diode-array detection and/or mass spectrometry is beginning to find wide application in the forensic toxicology laboratory as a screening tool. A primary limitation to the throughput of these methods has been the perceived need to equilibrate the column between runs with 10-20 column volumes of the initial mobile phase used in the gradient elution. Recently, the feasibility of reducing this equilibration step to less than 2 column volumes while maintaining acceptable repeatability in retention time was demonstrated [1]. By minimizing the HPLC (Agilent 1100) instrument dwell volume and maintaining precise cycle times we are able to screen blood extracts for drugs of abuse using a gradient elution method with an analysis time of 4.5 minutes per sample, including a gradient time of 2.5 minutes. Compound identifications are made by matching diode-array data (spectrum from 200-400 nm) and retention times against a spectral library constructed in-house using Chemstation software (Agilent). The average standard deviation in retention time for morphine, oxycodone, methamphetamine, benzoylecgonine, zolpidem, diazepam, alprazolam and amitriptyline was 1.2 seconds for 60 successive injections ranging from 2 to 200 ng of each drug, with an average relative standard deviation of 1.8%. Instrumental limits of detection ranged from 2 to 17 ng, with the lower limit for spectral matching (at 95% spectral match) being closer to 17 ng. This strategy is easily implemented on standard HPLC systems with minimal modification and serves to greatly increase laboratory throughput while reducing solvent waste.

[1] A.P. Schellinger, D.R. Stall, P.W. Carr. *J. Chromatogr. A.* 2005; 1064: 143-156. Key Words:

Screening, Drugs-of-abuse, LC

S38

Rapid Screening with the Direct Analysis in Real Time (DART) Mass Spectrometer System: Applications to Forensic Toxicology

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The objective of this paper is to present a new method for the rapid identification of unknown substances and for rapid quantitative screening of drugs in body fluids.

Direct Analysis in Real Time (DART) is a new ion source that permits rapid, non-contact analysis of gases, liquids, solids, or materials deposited on surfaces. The source was developed by James A. Laramee and one of the authors (Cody) during the years 2001 -2003, and was recently reported in the literature. DART allows mass spectrometric analysis to be carried out in open air at ground potential under ambient conditions.

In combination with a high-resolution time-of-flight mass spectrometer equipped with an atmospheric pressure ionization (API) interface, DART has been applied to the analysis of hundreds or thousands of samples ranging from licit and illicit drugs to explosives, chemical weapons agents and toxic industrial materials. Unknown substances can often be identified by a combination of exact mass measurements and accurate isotopic abundances. Fragment ions produced by varying the potentials in the API interface can often be used for confirmation.

We report here that DART can be applied to rapid quantitative screening for certain drugs in bodily fluids such as blood, saliva, or urine. An example is presented for the detection of the date-rape drug, gamma hydroxybutyrate (GHB) in urine. A deuterated internal standard was added to compensate for variations in sample handling. Untreated urine and synthetic urine samples were spiked with internal standard. No other sample preparation was required. Aliquots were applied to a glass melting point tube and placed without drying between the DART ion source and the mass spectrometer sampling orifice. The drug was detected within seconds as $C_4H_7O_3^-$ at *m/z* 103.0395. Standard samples containing varying concentrations of GHB were used to create working curves showing excellent linearity.

DART is complementary to other mass spectrometric methods of analysis, such as gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry LC/MS. Its principal advantages are that it requires little or no sample preparation, it produces simple mass spectra and is relatively insensitive to certain contaminants, and that samples can be analyzed very rapidly. Its principal limitation is the requirement that the analyte must be clearly distinguished from background interferences at the expected concentration levels, hi certain cases, such as the GHB analysis, this can be accomplished by relying on high resolution and/or compound-specific fragmentation.

Key Words: GHB, DART, screening

S39

Opioid Mortality in Southwestern Virginia

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Objective: A review of medical examiner records from southwestern Virginia, where there has been a significant increase in prescription drug deaths, comparing those records/ cases where opioid versus non-opioid prescription drugs were identified.

Over the last 6 years, the Office of The Chief Medical Examiner (OCME), Western District of Virginia, has documented a three-fold increase in prescription drug-related mortality in the southwestern region. These deaths involved poly-drug intoxications of prescription drugs with minimal involvement of the traditional "street drugs of abuse" (e.g., heroin, cocaine). To describe this trend, death certificates and toxicological results from OCME were analyzed. Oxycodone, methadone, and fentanyl were identified as predominant in these decedent cases and significant shifts emerged in the opioids identified over the time period. In 1998, 12% of the 69 certified drug deaths implicated opioids (3 oxycodone, 5 methadone, 0 fentanyl cases). By 2003, 70.8% of the 157 certified drug deaths implicated opioids (47 oxycodone, 88 methadone, 22 fentanyl cases).

Methods: Two hundred and eleven of 575 total certified drug-related decedent records from 1998-2003 were reviewed. Abstracted information included demographic data, medical and surgical history, social history, drugs identified by toxicological analysis, drug abuse history, and psychiatric history. Results were stratified to compare decedents in which opioids were identified as a direct or contributing cause of death ("opioid decedents") to those cases in which opioids were not identified ("non-opioid decedents"). Both suicide and accidental deaths were included.

Results: Decedents included 134 males and 77 females, with 208 Caucasians and 3 African-Americans. In less than 5 cases, heroin was identified as a direct or contributing cause of death. A comparison of the opioid decedents to the non-opioid decedents is highly suggestive of a systematic difference between the two groups. The opioid decedents were more likely to be male and have less education, more likely to die accidentally rather than by intent or suicide, more likely to have a history of treatment for pain, but were less likely than the non-opioid decedents to have been treated for mental illness. Opioid decedents were more likely to have been treated for drug abuse, but there was no significant difference in rates of alcoholism, chronic illness, or history of treatment for depression between the two groups.

Significance: Despite documented increases in rural prescription drug abuse in the US (SAMHSA 2004), descriptions of this phenomenon are lacking in the literature. This record review portrays prescription drug overdoses in such a rural population, contrasts opioid and non-opioid cases, and characterizes the individual most at risk for overdose from prescription drug abuse.

Key Words: Prescription drug abuse

S40

Field Evaluations of an Onsite Oral Fluid Drug Screen — Oratect® II

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Oral fluid is gaining interest as an alterative matrix for drugs of abuse screening due to ease of collection and reduced chance of adulteration. A new version of an onsite one-step device called Oratect® II has improved flow characteristic, a shortened test time of 5 min. and lower cut-off concentrations (Δ9-THC 40 ng/mL; Cocaine 20 ng/mL; MDMA/methamphetamine 25 ng/mL; amphetamine 25 ng/mL; opiates 10 ng/mL and PCP 4 ng/mL). Furthermore, the collection pad portion of the Oratect II can be sent to the laboratory for GC/MS analysis. To evaluate the performance of Oratect II in the field, a total of 222 subjects at a rehabilitation center were tested over four days with both the Oratect and the Intercept® oral fluid test device (Orasure Corp. For Oratect), 61.3% of the test subjects completed the collection process within 3 min. and 32.7% completed in 5 min. Overall, 22 subjects were tested positive with either Oratect or Intercept devices and the result is summarized below. Positive Intercept results were further analyzed by GC/MS procedure. Concentrations are shown in ng/mL.

Specimen No.	Drug detected by Oratect	Drug detected by Intercept	Specimen No.	Drug detected by Oratect	Drug detected by Intercept
1	Coc	Coc at 20	12	Amp	Amp at 200
2	Coc	Coc at 12	13	Opi	Opi at 8
3	Coc	Coc at 10	14	Opi	Opi at 8
4	Coc	Coc at 504	15	Opi	Opi at 82
5	Met	Met at 2700	16	Opi	Opi at 136
6	Met	Met at 90	17	Opi	negative
7	Met	Met at 190	18	Opi	Opi at 142
8	Met	Met at 370	19	Opi	Opi at 66
9	Amp	Amp at 530	20	Opi	Opi at 62
10	Amp	Amp at 70	21	Negative	THC at 5
11	Amp	Amp at 34	22	Negative	THCatll

Among the positive specimens, Oratect results correlate well with Intercept except in one opiate case and when THC concentrations were lower than the Oratect cut-off level. The data suggest that Oratect II is a viable oral fluid drug screen device.

Key Words: Oral fluid; Onsite drug screen; Oratect

S41

Molecular Autopsy with pharmacogenomics - A Multi-Center Study for Certifying Methadone Deaths: Preliminary Findings of Data Acquisition and Multiplex Genotyping *CYP 450 2D6, 2C9,2C19,3A4 and 3A5* by Pyrosequencing™

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Methadone is used for the addiction treatment of heroin and other opioids (such as oxycodone), and for pain management. Methadone intoxication due to abuse and diversion has recently increased in selected regions of the United States. The multi-pathways phase I metabolism of methadone is partially mediated by CYP 3A4, 2C9, 2C19, and 2D6. These are encoded by their respective polymorphic genes. A previous preliminary study of methadone death certification showed the prevalence of *CYP 2D6*3, *4* and *5 mutations higher than that of general population and that of a control group. However, the study was not adequately powered to reach statistically significant conclusion. Hence, a multi-center study was organized to enroll an adequate number of methadone cases to evaluate the potential contribution of genetic variation to methadone related deaths - Pharmacogenomics as an aspect of Molecular Autopsy. Personnel of medical examiner/coroner offices and several academic departments formed FPTPMSG. The study was approved by IRB. The study period was 2002 and 2003. Inclusion criteria were: cases certified with methadone toxicity, methadone related and methadone present. Whole blood samples were mailed to a central pharmacogenomics laboratory for multiplex genotyping for *CYP 2D6, 2C9, 2C19,* and *3A4/5* by Pyrosequencing™. By using a combination of Microsoft ACCESS and Excel database programs, case history and toxicology results were entered, followed by statistical analysis of transformed Excel data. With the current analysis of some of the samples, the following prevalence were identified in 93 samples: for *2D6 - *3,* 1.6%., **4,* 14.0%., *5, 5.4%., **6,* 1.6%., *7 and **8,* 0%., for *2C9 - *2* and **3,* 8.1% and 7.0%., for *2C19 - *2,* 13.4%, **3* and **4,* 0%., for *3A4*1B,* 5.9%., and *3A5*3,* 89.2%. The early results of the study demonstrated the feasibility of coordinated planning and samples collection, and initial data entry and transfer to the central pharmacogenomics laboratory via Internet by 5 centers. Further, multiplex genotyping by Pyrosequencing™ had been reliably performed with the previously collected postmortem whole blood samples.

Key Words: Methadone, pharmacogenomics, molecular autopsy

S42

Sensitive Identification, Screening and Confirmation of Drugs of Abuse in Blood Using Ion Trap and AP-OA-TOF LC/MS Techniques

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Several classes of abused drugs were analyzed using both ap-Ion Trap and ap-oa-TOF mass spectrometers in order to show how these complimentary techniques when combined can be an extremely powerful tool for their unambiguous identification and quantitation. MS" library searching together with automatically-generated accurate mass empirical formulae and database searches for identificative screening purposes will be demonstrated which can provide valuable scientific evidence for presentation to a court of law. Smart-fragmentation ramping and data-dependent acquisition for unknown samples using the ap-Ion Trap provides consistent MS" fragmentation spectra, even at low concentrations, providing a reliable screening and library search method. Quantitation linearities and sensitivities of both ap-Ion Trap and ap-oa-TOF are also outlined in this paper for each class of abused drug analyzed in this study.
Methodologies: Chromatographic MS" and accurate mass data was obtained using the Agilent LC/MS Ion Trap (XCT *Plus)* and the Agilent ap-TOF, respectively, in positive mode ESI, by injecting various columns using various gradient profiles. Mobile Phase A - 2mM aqueous ammonium acetate, Mobile Phase B - 100% acetonitrile, Injection Volume - Various (uL) Column Temperature 22 °C. Chromatographic MS" conditions were optimized for each of the compound classes by infusing standards at a rate of 600 |lL/hr with mobile phase set at 50:50 and pumped at 0.4 mL/min. In the case of ap-TOF, a series of automated flow injection analyses were used to indicate appropriate fragmentation and capillary voltages. v :
Typical MS ESI source settings were as follows:

Positive mode ESI	(Dual ESI source for oa-TOF data acquisition with reference spray)
Drying Gas Temp:	325 °C
Nebuliser pressure:	60 psi
Drying gas flow:	12L/min

Outline Results: This study has demonstrated that low levels of a variety of classes of abused drugs (plus metabolites) can be detected and identified at low concentrations from blood-derived samples. Cannabinoids, benzodiazepines, opiates and LSDs are reported and identified from the two mass spectrometers some of which show sensitivities in ESI mode at sub-ng/mL levels. The ion trap shows its suitability in MSn modes for production of high-quality and consistent fragmentation spectra by utilizing fragmentation ramping routines (Smart Frag). The quality of the MSn spectra is of utmost importance when searching against predefined libraries so that ion ratios and other confirmatory "fits" yield a realistic ID. This is demonstrated. The routine accurate mass data produced by the Agilent ap-TOF can be automatically searched with Empirical formulae data analysis routines to produce predicted empirical formulae for detected peaks in a mass chromatogram. Mass accuracies, together with isotopic match (intensities and accurate mass) are all used to provide a probability fit for the unknown, which can also be searched against a database of compounds for identification purposes. Spectral libraries are not needed with such databases, which depend on compelling accurate mass data with high mass tolerance. Combining the data from both MS techniques, therefore, shows unambiguous confirmation of drug analytes whether in quantitation or screening modes. Quantitative linearities over three orders of magnitude are illustrated for analytes, typically with correlation values of 0.998 or above.

Key Words: LCMS, Ion Trap, TOF, drugs of abuse

S43

Detection of Corticosteroids, Anabolic Steroids and b2-agonist in Athens Doping Control Lab, during Athens 2004 Olympic Games using Ion Trap Mass Spectrometers

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Anabolic steroids, Corticosteroids and B2-agonists are considered performance-enhancing drugs, intentionally regulated by most international sports agencies for the purpose of maintaining fairness in competition. Controlling the use of such drug usage among athletes should also be considered a health benefit. During the two weeks of the 2004 Summer Olympics in Athens, Greece, several ion trap instruments were used 24 hours per day for the analysis of extracted urine samples, meeting the World Anti-Doping Agency (WADA) guidelines for minimum required performance limits (MRPLs). In this presentation Agilent 1100 Series HPLCs are coupled with LC/MSD Trap SLs, each using an electro-spray ionization source (ESI) for the analysis of controlled substances. The MRPLs of 30 ng/mL urine for the anabolic steroids, 10 ng/mL urine for the Corticosteroids and B2-agonists are easily met. For example, 1 ng/mL urine for triamcinolone acetonide and less than 0.5 ng/mL for the designer drug tetrahydrogestrinone (THG). Moreover, the instrument sensitivity is shown to be maintained for the entire two weeks of competition even though the sample load was extensive.

Key Words: LCMS, Ion Trap, sports doping

S44

Zolpidem Impaired Drivers in Wisconsin - A Six Year Retrospective

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Zolpidem is a non-benzodiazepine, sedative hypnotic prescribed for short term treatment of insomnia. Administration is to coincide with a minimum of eight hours of uninterrupted sleep and users are cautioned against operating heavy machinery or motor vehicles. While unrelated to benzodiazepines in structure, zolpidem possesses similar pharmacological properties, acting as an .1-agonist through selective affinity with the $GABA_A$ receptor. Resulting impairment is similar to that caused by ethanol and other CNS depressants, with occasional reports of bizarre behavioral changes including agitation and depersonalization.

Detection of drugged drivers remains a key component of efforts to maintain safe roadways in Wisconsin. Of special interest is detection of those drivers who are impaired by drugs other than ethanol. During calendar years 1999 - 2004, the Wisconsin State Laboratory of Hygiene (WSLH) Toxicology Section performed ethanol and drug testing on 8,121 blood specimens submitted by state law enforcement agencies in suspected driving under the influence investigations. Zolpidem (Ambien™) was confirmed in 187 (n = 107 males) (n = 80 females) cases.

Based on single doses of 5 and 10 mg, peak serum concentrations occur approximately 1.5 hours post dosing and range between 29-272 ng/mL. In 60% of the study cases, blood zolpidem levels were 51-300 ng/mL, within the expected therapeutic range. Ethanol was detected in 23% of the cases. Poly drug use was common, with one or more drugs other than ethanol detected in 86% of the cases (1 drug = 35% and 2 or more drugs = 51%). While no clear pattern of use emerged, the benzodiazepine class was reported in 42 cases, carboxy-THC 30 cases (delta-9-THC n = 19), hydrocodone 27 cases and propoxyphene 18 cases.

This review examined in detail the 21 cases out of 187 where ethanol was not detected and zolpidem was the only drug present. Among these, 5 cases included an evaluation by a drug recognition expert (DRE). Incident reports from all 21 cases were compared in a number of categories, including sex, age, time of driving, signs/symptoms of driving impairment and zolpidem concentration. Zolpidem levels in 57% of these cases were in excess of expected single dose peak concentrations. The range of concentrations for males (n = 15) was 60-1200 ng/mL (mean = 414, median = 270) and females (n = 6) was 220-1100 ng/mL (mean = 580, median = 535).

Information noted by officers varied in detail, but certain similarities emerged. Drivers under the influence of zolpidem typically traveled well below posted speed limits and demonstrated difficulty with time perception. Subjects commonly traveled across multiple lanes of traffic, including over medians. Zolpidem impaired drivers were frequently involved in multiple hit and run events, before ultimately contacting a stationary object such as a traffic pole. Individuals had difficulty keeping their eyes open, maintaining balance, and following instructions, many of which were repeated multiple times. Sudden mood swings, from co-operative to aggressive, were documented in two of these cases as well. Individual case studies will be used to highlight some of the more interesting observations.

Key Words: zolpidem, driving, impairment

S45

A New Collection Tool as Basis for a Practicable Drug of Abuse Monitoring in Saliva

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Introduction: Saliva has a number of advantages as sample matrix in drug of abuse testing: it is easy available and non-invasive. But sampling of saliva still encompasses difficulties like xerostomia or analyte discrimination due to unspecific adsorption of analytes on/in many collection system. One further obstacle is the rather cumbersome and irreproducible sample collection and handling. Referring to SAMSHA criteria, which specify cut off values for drug testing in saliva, an urgent need for a platform which allows universal application of the analyte media saliva is required.
Objectives: Based on the company's experience in the field of preanalyses, a new practicable tool for saliva collection has been developed by Greiner Coop, through the Vacuette® Saliva Extraction System (SES). The SES system contains a non-toxic, coloured saliva collection solution, a collection beaker and a evacuated tube. Saliva is collected by keeping by the tested person the non-toxic saliva collection solution in the mouth for a short period of time. The solution is then directly voided into the collection beaker. With the Vacuette® collection tube this solution can be hygienically and directly transferred into a closed storage / transport tube. Additives in the tube help to precipitate mucous material and to stabilize the saliva solution. After centrifugation, a clear supernatant solution is available for many applications in drug of abuse testing or therapeutic drug monitoring. The exact volume of the collected (dissolved) saliva in the SES solution can be easily determined by measurement of the absorption change of the non-toxic dye in the collection solution.
Methods and Results: The practicability of the saliva collection system was tested in a study with volunteers after (licit) consumption of poppy seeds.
Twelve volunteers consumed commercially available (morphine-containing) poppy seeds. Blood samples were drawn together with saliva collected by the Greiner SES system at different pH values during the following 8 hours. Morphine was measured by a modified CEDIA opiate assay on a Hitachi 912 clinical analyser and confirmed by LC-MS. It was found that morphine (and codeine) raised significantly depending on the amount of poppy seeds consumed in blood up to 300ng/mL and in saliva up to lOOng/mL. Parallel to this in saliva morphine and codeine followed the values in blood, but the kinetics of morphine in saliva turned out to be shorter than for blood. Depending on the pH in the saliva collection solution the saliva/serum ratios increased parallel to the increase of pH in the SES solution.
Conclusions: The non-invasive saliva collection method system SES has major advantages over other available systems: Even in cases of reduced saliva production also small saliva volumes may be utilized precisely and reproducible. By using the same closed sample tube system during saliva collection as for blood collection, handling of saliva samples becomes very hygienic and any infection risk is reduced to a minimum. After centrifugation of the SES saliva samples tubes they can be directly introduced and automatically handled by most (all) common clinical analyser systems. These results confirm the applicability of the Vacuette® Saliva Collection System for the practical use of saliva as an analyte matrix in drug screening.

KeyWords: Saliva, DOA testing, morphine, poppy seeds

S46

Percutaneous Absorption and Distribution of Methanol in a Homicide

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ABSTRACT: A woman was alleged to have committed suicide by consuming a gasoline additive shortly before jumping from a second floor balcony within her home. She was found dead by police with a multitude of injuries, lying nude in a partially evaporated unknown residue that was later determined to be methanol. Samples collected at autopsy were found to contain methanol in the following concentrations: femoral blood 31.2 mg/dL, pulmonary artery blood 111.0 mg/dL, aortic blood 77.8 mg/dL, vitreous fluid 196.4 mg/dL, brain 22.0 mg/lOOg, liver 21.2 mg/lOOg and kidney 25.9 mg/lOOg using a headspace gas-chromatographic method. Significantly, no methanol was detected in samples recovered from the esophagus, stomach, duodenum, small intestine, bile or urine. These findings are inconsistent with either recent or delayed oral ingestion of methanol. We conclude that absorption of methanol occurred dermally and through the oral mucosa as she lay dying, saturated in the fuel additive. Using previously published percutaneous absorption rates for methanol, we calculate that the estimated dose could be absorbed in approximately 7-12 minutes. Based upon the toxicologic data and a comprehensive forensic investigation (including documentation and analysis of evidence recovered at the scene and the autopsy), the cause of death was determined to be blunt impact trauma and methanol poisoning.

Key Words: methanol, wood alcohol, percutaneous absorption, homicide.

S47

Fatal Oral Ingestion of a Large Amount of Cocaine By a One Year Old Child

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The case history and toxicological findings of a child fatality are presented. The decedent is a one year-old African American female child who reportedly has had a recent bout of pneumonia. The parents reportedly put the decedent and her two year-old sibling to bed and then they smoked marijuana and went to bed. The parents went to sleep and awoke a short time later to the sound of water running in the bathroom. The father went into the bathroom and found the decedent with a white powder around her mouth at which time he tried to rub it off, thereby causing her tongue to bleed. The decedent's mother then intervened and placed the child into a playpen but began to notice that the child was acting unusual. At this time the parents transported the decedent and her sibling to Phoenix Children's Hospital by private car where death was pronounced. The decedent's father admitted to hospital staff that there was cocaine in the house and indeed the sibling likewise tested positive for cocaine while at the hospital but survived.

An autopsy by an assistant medical examiner revealed in this otherwise normal sixteen pound child the presence of cerebral edema, pulmonary congestion and edema, cocaine "rocks" in the stomach, bilateral hydro-thoraces and ascites. No virus was isolated from appropriate cultures and the metabolic screen for inborn errors of metabolism was negative. Because of the positive cocaine findings, the case was signed out as a cocaine overdose and the manner of death was homicide.

A comprehensive testing protocol was utilized on cardiac blood, bile, pleural fluid, brain, liver, spleen, kidney and gastric contents consisting of ELISA screening, GC-NPD, GC/MS confirmation and then subsequent GC/MS quantification with a five-point calibration curve by SPE extraction to give the following results:

Specimen	Concentrations (mg/L or mg/kg)		
	EME_____	BE_____	Cocaine
Cardiac Blood	10.13	4.82	11.54
Bile	14.00	6.17	42.23
Pleural Fluid	9.49	4.32	11.75
Brain	9.69	3.27	25.30
Liver	22.34	9.64	33.28
Spleen	14.96	6.94	78.11
Kidney	36.07	26.15	220
Gastric	136	>900	>2000

To our knowledge these are some of the highest concentrations seen in other than body-packer cases. While there are certainly limitations on interpreting postmortem cocaine levels in blood and other tissues, especially in the context of limited data on children, we believe these values support a cocaine overdose. Our conclusion is based upon the age of the child, the negative autopsy findings and the levels of drug found. The manner of death was classified as a homicide. AEME also was detected in the blood/gastric "rocks".

Key Words: Cocaine, Postmortem Toxicology, Pediatric Toxicology

S48

Measurement of Nicotine and Cotinine in Hair of Children and Adults by GC/MS/MS

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The amounts of nicotine and cotinine in head hair samples were compared with self-reported histories of tobacco use and exposure to cigarette smoke in a population of 88 adults and 126 children (ages 1-17 years, median age 5 years). For analysis, 12 mg hair samples, collected in a medical setting, were aggressively washed (6 washes, 3.75 hours) and then digested in 1 mL of 45% NaOH at 70 oC for one hour. Using an isotope dilution protocol, nicotine and cotinine were separated by Solid Phase Extraction followed by capillary gas chromatography/mass spectrometry/mass spectrometry (GC-MS-MS) utilizing a Finnigan TSQ 7000 triple quadrupole instrument operating in positive chemical ionization (PCI) mode. The linearity of the method for nicotine and cotinine was from 1-120 ng/10 mg hair and from 0.10 - 50 ng/10 mg hair, respectively. The TSQ operated in PCI with both Ql and Q3 set at unit resolution. The target parent ions for nicotine and its internal standard nicotine-d-4 and for cotinine and its internal standard cotinine d-3 in Ql were m/z 163 and 167, and 177 and 180, respectively. The target ions for product or daughter ions for nicotine, nicotine d-4, cotinine and cotinine d-3 in Q3, were m/z 84, 84, 80, and 80, respectively.

The extent of smoking in the smokers' group ranged from 1 to 350 cigarettes/week. The range of nicotine levels in hair of 40 adult smokers was 0.33 - 714, with a mean of 53.9 (S.D. 117) and a median of 29.6. In comparison, non-smokers' hair nicotine values ranged from 0.16-21.7 ng/10 mg hair, with a mean of 2.09 (S.D. 4.38) and a median of 0.43. Five values ranging from 6.69 - 21.7 for non-smokers may have been outliers (perhaps smokers); excluding these, the mean nicotine value for non-smokers was 0.75 ng/10 mg hair (SD 0.81, median 0.35). Forty of 48 self-reported non-smokers had cotinine values of 0, five had values of .039 - .09, and three had values from 0.11 to 0.17. (The same subjects with higher nicotine in hair also had the higher cotinine values). This contrasts sharply with cotinine in smokers' hair, where one subject who smoked 1 cigarette per week had no cotinine in the hair, and the others contained cotinine ranging from 0.11 -12.2 ng/10 mg hair. However, there was little direct correlation between the number of cigarettes smoked and measured nicotine or cotinine levels. This may be due to the known inaccuracies of self-reports and to the variable nicotine content of different cigarette brands. Nicotine levels in the hair of 101 children who were reported to live in smoke-free environments were 0.07 to 5.90 ng/10 mg hair (mean 0.75, S.D. 1.04, median 0.37). Cotinine in the hair of these subjects ranged from 0 to 0.31 (mean 0.019, SD 0.06, median 0). Nicotine levels in 25 children reported to be exposed to smoke had hair nicotine levels of 0.16-15.3 ng/10 mg hair (mean 1.66, SD 3.14, median 0.64). Cotinine levels in this group were 0 - 0.24 ng/10 mg hair (mean .019, SD .051, median 0). The results suggest that nicotine alone may be too ubiquitous in the environment to use alone as a marker for smoking. Highly sensitive measurements of cotinine in hair appear to be a more reliable indicator of cigarette smoking, as the levels were much lower in the non-smokers. Measurement of both analytes, in well washed hair, is likely to provide the most effective indicator of nicotine ingestion.

Key Words: Nicotine, Cotinine, Hair

S49

LC/MS/MS Analysis of THC and its Metabolites

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Objective

Cannabis (marijuana) is the most commonly used illicit drug. Δ9-Tetrahydrocannabinol (THC) is the active compound in cannabis and its major metabolites are 11-hydroxy- Δ9-tetrahydrocannabinol (11 -OH-THC) and 11 -nor-9-carboxy- Δ9-tetrahydrocannabinol (THC-COOH). Because of its prevalent use, there is an increased demand for detection and quantification of THC and its metabolites in toxicological assays. Until recently, screening has been accomplished by immunoassay and quantification utilizing GC/MS. Over the past 10 years, use of LC/MS/MS has significantly increased in many analytical areas, including toxicology. LC/MS/MS often achieves better detection limits versus GC/MS and sample preparation is less labor intensive. A quick and rugged method for analysis of THC and its major metabolites was developed using a hybrid triple quadrupole/linear ion trap LC/MS/MS system.

Methods

THC and its metabolites were analyzed using LC/MS/MS. A hybrid triple quadrupole/linear ion trap mass spectrometer, which has the capability to acquire qualitative and quantitative data in a single experiment, was used. APCI and ESI in both positive and negative modes were investigated to determine which mode exhibited the best signal-to-noise; ESI negative yielded the best results.

Liquid-liquid and solid-phase extractions, as well as protein precipitation techniques, were studied for extraction of the analytes from whole blood and post-mortem tissue. Extraction efficiencies for all methods were greater than 75%. Protein precipitation was utilized because of its simplicity.

Results

Detection limits for all analytes were less than 0.1 ng/mL and the reproducibility and ruggedness was shown to be extremely good. Accuracies were within about 10% and RSDs were generally ≤5%, increasing up to 10% at levels close to the quantitation limits. Matrix effects were either not present or insignificant.

Conclusion

An LC/MS/MS technique for extraction, detection, and quantification of THC and its metabolites was developed. This technique showed excellent precision and accuracy and improved detection limits versus GC/MS. Sample preparation was also greatly simplified versus GC/MS analysis, especially since no derivatization was required. Run times were less than 10 minutes, which further reduced the overall analysis time. The ability to acquire both qualitative and quantitative data in a single assay allowed for detection, confirmation, and quantification in a single run.

Key Words: LC/MS/MS, THC

S50

Chloroform and Hydrochloric Acid Identified in Human Remains

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A homicide as a result of Chloroform inhalation and subsequent immersion in a barrel of Hydrochloric Acid was investigated. The detection of Hydrochloric Acid in human kidney, liver, muscle, and adipose tissues was accomplished by colorimetric chloride levels in combination with pH measurements, while the measurement of Chloroform and Ethanol were accomplished by head space GC/MS. Drug screening was performed by RIA and confirmations performed by GC/MS. The tissues revealed positive traces of Hydrochloric Acid.

The Chloroform measurements were: kidney 11.0)J-g/g, liver 36.4 |ig/g, muscle 10.2, adipose 25.3 |ig/g. In addition, the ethanol content of the kidneys ranged .03-.04g%, liver .06g%, and muscle .1 lg%. No other drugs were confirmed by GC/MS.

The cause of death was listed as probable combined effects of Acute Chloroform exposure and Hydrochloric acid immersion.

Key Words: Chloroform, Hydrochloric Acid, GC/MS

S51

Direct Multistage Mass Spectrometric Method for Identification of Controlled Substances in Urine

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The aim of the study was to develop simple and rapid mass spectrometric assay methods for forensic identification of controlled substances in urine. Mass spectrometry has long been used for the unequivocal identification of organic molecules. Multistage mass spectrometry provides additional compound-specific structural information, although there are limitations in the analysis of structurally-related isomers. Our current work is to determine if the specificity of multistage mass spectrometry can be taken advantage of to eliminate time consuming chromatographic sample purification steps. We have developed and applied direct-injection multistage mass spectrometric methods to the identification of methamphetamine, 3,4-methylenedioxymethamphetamine and fentanyl in urine. Urine (1 mL) samples obtained at autopsy from overdose deaths were spiked with 10 µL of deuterium labeled internal standards (10 µg/mL), then filtered through a 0.2 jam PTFE membrane. Aliquots (50 µL) of the filtrates were pipetted into 1-mL polypropylene centrifuge tubes and diluted to 200 µL total volume with 0.1% formic acid in acetonitrile. Direct infusion (10 µL/min) into an ion trap MS operating in the positive ion mode with an electrospray ionization source. Multistage mass spectra recorded in MS, MS/MS and MS/MS/MS (MS^3) modes were used to confirm the presence of drugs in urine. Ion suppression did not hinder the analyses at analyte concentrations above 100 ng/mL of urine. Electrospray ionization multistage mass spectrometry without chromatographic separation steps was used to rapidly identify several drugs of abuse in the urine of overdose victims.

Key Words: Controlled Substance, Analysis, Mass Spectrometry

S52

A GC-MS Method Development Study for the Determination of Ketamine or its Metabolites and the Evaluation of the REMEDi Screening Method Using Real Urine Samples

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Urine drug screening by REMEDi HS system has been used as a monitoring tool for drug abuse in our laboratory. The objective of the study was to establish a gas chromatography-mass spectrometry (GC-MS) method for urine ketamine and its metabolites analysis. The method of REMEDi was then evaluated by comparing analytical results of ketamine (K), norketamine (NK) and dehydronorketamine (DHNK) in urine samples from suspected drug-user cases obtained in 2004.

Ketamine and metabolites in urine samples were extracted using automated solid-phase extraction then analyzed by GC-MS without further derivatization. This method showed good linearity (r^2 = 0.999) over the concentration range of 30-1000 ng/mL. The limits of quantitation were 15, 10, and 20 ng/mL for K, NK, and DHNK, respectively. High accuracy (90 %-104 %) and high precision (RSD < 8 %) was found for all analytes.

Ketamine or its metabolites were detected in 53 of the 240 urine samples. K, NK, and DHNK were detected simultaneously in 48 of these samples. DHNK concentration was generally the highest while K concentration was usually the lowest. Concentration ratios between K, NK, and DHNK were variable among individuals. According to the cutoff values set by Taiwan Department of Health (concentration of K, NK, or K+NK 100 ng/mL), fifty urine specimens were confirmed positive. The results, which supported this GC-MS method, can effectively and accurately identify ketamine positive urine samples. The broad-spectrum drug-screening instrument REMEDi HS system (BIO RAD Co.) is a high performance liquid chromatography device with a UV detector and a built-in spectrum library. Ketamine is among the target analytes detected by REMEDi. From the results of 206 of the same 240 urine samples analyzed by REMEDi, this screening method has high efficiency (95 %), excellent specificity (100 %), and 0 % false positive rate. However, because the K cutoff level is lower than the REMEDi limit of detection and REMEDi cannot detect NK or DHNK, low sensitivity (42 %) and high false negative rate (58 %) problems occurred.

Key Words: Ketamine, GC-MS, REMEDi

S53

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Abstract:

Pesticides increase the agricultural productivity and reduce the crop loss. Methyl parathion, an organophosphate insecticide and acaricide used to control boll weevils and many biting or sucking insect pests of agriculture crops, primarily on cotton, is widely used by farmers in South India to commit suicide. Ml wt. 263.20, with a Vapor pressure: 1.3mPa at 20°c and Solubility: 55-60 mg/L water at 25°c (pure compound), and soluble in most organic solvents like n-Hexane, dichloromethane, 2-Proponal, toluene. Inhibiting acetyl cholinesterase via methyl paraoxon, resulting in the stimulation of central nervous system, parasympathetic nervous system, and the motor nerves. The new method of isolation from different matrix was compared with the existing method.

Extraction and Isolation:

Different solvents like hexane, dichloromethane, 2-propanol, toluene were tried for the isolation and extraction of methyl parathion from biological matrices. The recovery of residue of methyl parathion was found excellent in toluene. lOOg of tissue was macerated in to fine slurry in tissue digester and transferred in to conical flask (250cc.) with solvents for overnight.

Extraction of pesticide from blood: l0mL of blood is mixed with 10 mL of 10% sodium tungstate solution and 15mL of 0.1 N. sulphuric acid. Shaken for two minutes and then filtered. The residue is washed twice with 15mL of 0.1N sulphuric acid. The washings are collected and mixed with the filtrate, transferred in to a separating funnel and extracted thrice with 20mL of toluene. The toluene layers are combined, passed through anhydrous sodium sulphate and the solvent removed by evaporation. Purified by back extraction in acetonitrile and hexane. Evaporated residue and reconstituted in toluene for analysis.

Carried out TLC with five different solvent systems as mentioned were tried and visualized with 2 reagents 1) Palladium chloride, 2) Bromination followed by Congo red and their respective Rf values are tabulated below. Silica gel -GF254 plates were used with mobile phase: 1) n- Hexane: Acetone (80:20) Rf-53, 2) Cyclohexane: chloroform (70:30), Rf 61, 3) Petroleum ether: Acetone ((70:30), Rf 63, 4) Benzene: Methanol, Rf 22, 5) Cyclohexane: Acetone: Chloroform (70:25:5) Rf 77. With spray reagents 1) Palladium chloride, yellow to brown coloured spots and 2) Bromination followed by Congo red, blue coloured spots with light pink coloured background.

Results and discussion:

The standard procedure was followed for the isolation and identification with different solvent systems 1,2,3,4, and 5. The solvent systems 1,2,3 and 5 were given better resolution and better Rf values. Confirmatory: GC-MS: The samples were qualitatively analyzed and the identity of the sample was confirmed by the mass fragmentation pattern of the samples compared with the standard Mass spectrum (NIST) from library.
Drawback: The insecticide methyl parathion was spiked to the tissues in the conditions from 0.1 mL to 1mL of 0.01 N acetic acid and 0.1 - 1 mL of 0.01 N ammonia solutions. Under these concentration ranges, the insecticide methyl parathion could not be detected in the visceral tissues.

Key Words: Methyl Parathion, GC-MS, standardization

ABSTRACTS

POSTER

PRESENTATIONS

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P1

Can an Immunoassay Become a Standard Technique in Detecting Oxycodone and its Metabolites?

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Opiate toxicology testing is routinely performed in the hospital setting to identify abusers and/or to determine those patients who are not taking prescribed opiate analgesics such as oxycodone. Commercially available assays for opiate detection in urine have decreased sensitivity for oxycodone, which contributes to a high false negative rate. Functioning as a beta site, our Veterans Affairs hospital evaluated a new enzyme immunoassay, DRI® Oxycodone Assay (Microgenics Corp: Fremont, CA), for its use in the qualitative and semi-quantitative detection of oxycodone in urine. We hypothesize that an immunoassay for oxycodone with superior sensitivity and specificity, when compared to the traditional opiate assays, would reduce the need for more expensive and time-consuming confirmatory testing.

We used the new liquid homogenous enzyme immunoassay to determine oxycodone results on a total of 148 urine samples from 4 different sample groups. Gas chromatography-mass spectroscopy was subsequently used to confirm the presence or absence of oxycodone (or its primary metabolite, noroxycodone). We also evaluated within run, between run, and linearity studies and conducted a crossover study to establish a cutoff value for oxycodone. In our patient population, we used the new DRI immunoassay to evaluate 17,069 urine samples to estimate oxycodone misuse profiles (patients not taking prescribed oxycodone or taking oxycodone without a prescription) during a 4-month period.

The sensitivity and specificity of the new oxycodone immunoassay were 97.7% and 100%, respectively at the cutoff concentration of 300ng/mL. The assay linearity was 1250 ng/mL, and the sensitivity was 10 ng/mL. Within run precision and between run coefficient-of-variations were 2.3% and 1.8%, respectively. None of the 15 compounds that we evaluated for interference had crossover significant enough to produce a positive oxycodone result when using 300 ng/mL as the cutoff value. None of the 17,069-oxycodone immunoassays were followed with a request for confirmation. Among patients with positive results (n=224), 93 (41.5%) were not prescribed oxycodone.

The new DRI Oxycodone Assay is a sensitive and specific screening test for the determination of oxycodone. The improved opiate screening results may lead to better patient and prescription management, to decreased laboratory spending, and to the identification of oxycodone abusers which could result in decreased oxycodone related mortality.

Key Words: Oxycodone, Immunoassay, opioid

P2

Solid Phase Extraction and Analysis of Clonazepam/7-Aminoclonazepam in blood and urine using a dual internal standard methodology

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In this presentation, a simple and robust method for the determination of clonazepam and its primary metabolite (7-aminoclonazepam) in blood and urine is described. Clonazepam (Klonapin) is a popular prescription drug that has been implicated in the field of drug facilitated sexual assaults (DPS A).

Clonazepam, 7-aminoclonazepam and the internal standards (deuterated analogues for GC-MS analysis and nitrazepam for analysis by LC-PDA/ GC-MS) were spiked into blood and urine samples. The samples were buffered with a pH 6 phosphate solution (5 mL) and extracted from phenyl SPE columns. The columns were washed with 5% acetonitrile in pH 6 buffer (3 mL) and eluted with ethyl acetate (2x 3mL). The eluents were evaporated for further chromatographic analysis. For GC-MS, the samples were derivatized prior to analysis, with LC-PDA the samples were reconstituted in distilled water.

From this method LOQ's of 5 ng/mL of sample is easily achievable by either chromatographic system. By using GC-MS in El mode, 1 ng/mL of sample can be detected.

Data is presented here to show the simplicity and efficiency of the extraction scheme. By employing the properties of GC-MS and LC-PDA, this extraction and analysis procedure further extends the number of tools open to the forensic toxicologist for the analysis of this drug.

Key Words: Clonazepam, SPE, Toxicology

P3

Urine pH, Container Composition, and Exposure Time Influence Adsorptive Loss of 11-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid

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1 l-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (1 l-nor-Δ^9-THC-COOH) is the primary cannabinoid present in the urine of individuals who have used marijuana and is the target analyte identified at forensic urinalysis drug testing laboratories. The preparation, storage, transport, and processing of control materials for gas chromatography - mass spectrometric analysis of human urine specimens is critical to accurate compound identification and quantification. Previous studies have suggested that adsorptive loss of 1 l-nor- Δ^9-THC-COOH is influenced by container composition and storage temperature.

In this study, urine solutions of 1 l-nor- Δ^9-THC-COOH (7.5, 15, 60, and 500 ng/mL) at three physiologically-relevant pHs (4.6, 6.5, and 8.4) were prepared and subjected to storage and processing in containers of different compositions (polypropylene and, borosilicate glass). Analyte identification and quantification was achieved using tetramethyl ammonium hydroxide / iodomethane-based derivatization followed by gas chromatographic separation and electron-impact mass spectrometry. These analyses demonstrate that adsorptive loss of 11-nor- Δ^9 -THC-COOH is a phenomenon found in acidic urine solutions and is relatively absent in urine solutions that are near-neutral or basic. Furthermore, the data indicates that the adsorptive loss of 1 l-nor- Δ^9-THC-COOH is dependent on solution / container exposure time and is similar between containers of two distinct compositions. These results suggest that for optimal analytical control performance, solution pH, and control processing times are critical elements.

Key Words: THC, Adsorption, pH.

P4

Determination of Ephedrine / Pseudoephedrine in Over the Counter Medications by Gas Chromatography

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Ephedrine/pseudoephedrine (EP/PSE) is commonly used as the starting product precursor, in the manufacture of methamphetamine by clandestine laboratories. The amount of starting product, EP/PSE, which is available for methamphetamine production, is commonly used in the sentencing guidelines of criminals who are convicted of clandestine methamphetamine production. Knowing the exact amount of EP/PSE, which is available and is extracted from over the counter formulations is therefore a valuable piece of information in the calculation of methamphetamine production. We present a simple, rapid, selective, direct procedure for the quantitation of EP/PSE in over the counter medications. The advantage of this method is that no derivatization is required prior to gas chromatographic (GC) analysis. Pills containing PSE were dissolved in solvents, which are commonly used by clandestine methamphetamine laboratories. In order to see how much of the drug would be extracted into these solvents at a certain time interval, an aliquot of the solvent (diluent) was withdrawn at one hour and was analyzed for PSE by gas chromatography (GC) with a flame ionization detector (FID). We used a coiled glass column, 1.2 m x 2 mm ID, packed with 3% SP-2250 Supelcoport 80/100 mesh (Supelco, Bellefonte, PA, 16823). Diphenhydramine (DH, 5 mg/mL) is used as internal standard (IS). To 0.1 mL of sample add 0.2 mL of IS. Add 1 mL of 0.5 M borate buffer and 1 mL of chloroform/isobutanol (98/2 by volume). Vortex, mix, and then centrifuge for 5 minutes. Aspirate top aqueous layer. Transfer bottom layer to small 12 X 75 mm glass tubes and evaporate to dryness. Dissolve the residue into 100 microliters of methanol and inject one microliter on the GC column. Helium is used as carrier gas at 30 mL /min. Gas Chromatography conditions were: injector 250°C, detector 300°C, column 165°C for 1.5 min. programmed at 70°C per min. to 280°C for 3 min. Retention time for ephedrine is 0.23 relative to DH, 3.86 minutes. Excellent linearity was observed in the 1.0 to 2.0 mg/mL range (slope = 0.21, y-intercept = 0.00, correlation coefficient = 1.0). Non linearity was observed above 2 mg/mL. Within-run precision is 12 % at 1.0 mg/mL. Between-run precision is 7 % at 1.0 mg/mL and 15% at 2.0 mg/mL. Percent recovery is 102 ± 7% at 1 mg/mL, 95 ± 15% at 2 mg/mL. Analyses of over the counter PSE tablets indicate that chlorpheniramine, triprolidine and other common medications do not interfere with this procedure. Preliminary data indicate that the amount of PSE as stated by the manufacturer on the package label should be verified. We conclude that a simple, rapid, sensitive and inexpensive method has been presented for the identification and quantitation of EP/PSE in over the counter medications. The sensitivity of the method is less than 0.1 mg/mL.

Key Words: ephedrine/pseudoephedrine, analysis, tablets

P5

A Comparison of Confirmatory Methods for the Quantisation of Benzodiazepines in Whole Blood or Urine

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There are a number of methods for the extraction and identification of benzodiazepine (BZD) drugs in serum, whole blood, and urine. Since BZDs have pharmacologically active metabolites, analytical methods must have different limits of detection to simultaneously identify the target compound and its metabolites. We have compared three extraction methods for BZDs in blood and urine: i) liquid-liquid extraction, ii) Clean Screen (United Chemical Technologies, Inc.) solid phase extraction and iii) Strata Cation Mixed-Mode Polymer solid phase extraction. Both GC/MS (with and without derivitization) and reversed phase HPLC were used for detection. We will present the results of the different extraction and detection methods. The figures of merit for each method including limits of detection, recovery, and sensitivity will also be presented. The accuracy of each method was verified using a certified serum containing five BZDs: diazepam, flunitrazepam, oxazepam, bromazepam, and lorazepam and two metabolites: 7-aminoflunitrazepam and desmethyldiazepam. Following a discussion of the advantages and disadvantages of the different extraction and detection methods a final recommendation will be made.

Key Words: benzodiazepines, HPLC, extraction

P6

Evaluation of the Abbott AxSYM Opiate Immunoassay With Modified Cut-off Calibrations of 100 ng/mL and 50 ng/mL For The Detection of Opiates In Urine

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The Abbott AxSYM Opiate fluorescence polarization immunoassay (AxSYM Opiate Assay) applying the manufacturer's recommended cut-off value of 300 ng/mL, is known to have poor cross-reactivity with various semi-synthetic opiates, particularly the widely prescribed oxycodone and oxymorphone.

Objective: To evaluate the detection of opiate drugs in urine by the AxSYM Opiate Assay applying morphine cut-off calibrations of 100 ng/mL and 50 ng/mL. Methods: One thousand five hundred twenty three urine specimens collected from pain management patients were tested for opiates with the AxSYM Opiate Assay, and gas chromatography/mass spectrometry. Immunoassay reagents were employed as directed by the manufacturers with the exception that a cut-off calibrations of 100 ng/mL and 50 ng/mL were applied for the detection of opiate drugs. All specimens were also analyzed by a GC/MS SIM procedure that employed treating the urine with hydroxylamine, a solid phase column extraction, and formation of the BSTFA derivative of the opiate analytes. The method detected codeine, hydrocodone, hydromorphone, morphine, oxycodone and oxymorphone. The limit of detection and the lower limit of quantification was 20 ng/mL for each opiate.

Results: Of the 1523 specimens tested by the AxSYM Opiate Assay applying a 100 ng/mL cut-off concentration, 974 specimens tested positive and 549 specimens tested negative. Of the 974 positive test results, 972 were confirmed positive for opiates by GC/MS; yielding an analytical selectivity at the 100 ng/mL cut-off of 99.5%. Of the 549 negative test results, only 381 were confirmed negative by GC/MS. Therefore, the AxSYM Opiate Assay at a 100 ng/mL cut-off yielded an analytical sensitivity of only 85%. However, when a 50 ng/mL cut-off was applied, 1134 of the 1523 specimens tested positive by the AxSYM Opiate Assay and 389 yielded negative results. Of the 1134 positive test results, 1118 were confirmed positive for opiates by GC/MS; yielding an analytical selectivity at 50 ng/mL cut-off of 95.8%. Of the 389 negative test results, 367 were confirmed negative by GC/MS. The vast majority of the false negative results were with specimens containing oxycodone and/or oxymorphone at concentrations of 100 to 200 ng/mL. However, two specimens yielding false negative results contained approximately 20,000 ng/mL of both oxycodone and oxymorphone. By using a 50 ng/mL cut-off for the AxSYM Opiate Assay, the analytical sensitivity increased to 98% and the concordance of positive and negative results with GC/MS was 97.5%.

Conclusion: The AxSYM Opiate Assay at a 50 ng/mL cut-off has sufficient analytical efficiency for the detection of opiates in most urine drug testing applications. If a zero tolerance for false negative results is required, only specimens testing negative by the AxSYM Opiate Assay need be further screened by a specific oxycodone immunoassay.

Key Words: AxSYM, Opiates, Immunoassay, Urine Drug Testing

P7

Simultaneous Quantitation of Atenolol, Metoprolol, and Propranolol in Biological Matrices Via LC/MS

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Hypertension is a growing medical concern in the United States. With the number of Americans suffering from hypertension increasing, the use of antihypertensive medications is increasing as well, hi fact, three beta-blockers — atenolol, metoprolol and propranolol — were among the 200 most prescribed medications in the United States in 2003. Pilots that successfully manage their hypertension can remain certified to fly. The Federal Aviation Administration currently designates approximately 8% of active pilots as "hypertensive with medication." The Civil Aerospace Medical Institute (CAMI) performs toxicological evaluation on victims of fatal aviation accidents. At CAMI, beta-blockers are analyzed using gas chromatography with mass spectrometric detection. We have, however, recently developed a liquid chromatography with mass spectrometric detection (LC/MS) method for the simultaneous quantitation of three commonly prescribed beta-blockers: atenolol, metoprolol and propranolol. One advantage of this LC/MS method is the specificity provided by an ion trap MS. Utilizing an ion trap MS, we were able to conduct MS/MS and MS/MS/MS on each analyte. This method also eliminates the time-consuming and costly derivatization step necessary during GC/MS analysis. Additionally, by utilizing this novel method, any concerns about beta-blocker metabolite and/or sample matrix interference are eliminated. The limits of detection for this method ranged from 0.39 - 0.78 ng/mL and the linear dynamic range was generally 1.6 - 3200 ng/mL. The extraction efficiencies for each analyte ranged from 58 - 82%. This method was successfully applied to postmortem fluid and tissue specimens obtained from victims of three separate aviation accidents.

Key Words: Beta-blockers, LC/MS, Forensic Toxicology

P8

Simultaneous screening of 26 drugs in urine by Liquid Chromatography - Tandem Mass Spectrometry

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The objective of this study was to combine several analytical techniques in one methodology in order to speed-up and simplify screening procedures for 26 forensic drugs that are incorporated into a panel for Medical Professional drug testing. The described method of Liquid Chromatography - Tandem Mass Spectrometry (LC/MSMS) allowed the detection and quantitation of several antihistamines (doxylamine, pheniramine, chlorpheniramine, brompheniramine, diphenhydramine), antidepressants (amitriptyline, fluoxetine, nortriptyline, sertraline, ambient), narcotics (naltrexone, nalbuphine, ketamine, tramadol, pentazocine, butorphanol, fentanyl, buprenorphine, meperidine) and stimulants (phenylpropanolamine, pseudoephedrine, phendimetrazine, diethylpropion, phenmetrazine, phentermine, methylphenidate) in a single extract of urine sample. The same drugs were previously split into three groups and analyzed separately by immunoassay (ELISA), Gas Chromatography-Mass Spectrometry (GCMS) and Gas Chromatography with a Nitrogen Phosphorus detector.

Most of the presently known LC/MSMS methods demonstrate a multi-drug screening technique for matrices such as plasma and blood. Urine remains a very challenging matrix due to the high content of polar compounds that cause "matrix suppression" on LC/MSMS ion sources. Turbo Ion Spray source that is very effective in detection readily ionized basic drugs is also known to be susceptible to matrix suppression. In order to minimize this effect, a matrix clean-up was performed by cation-exchange solid phase extraction including an extensive column wash with water followed by acetic acid and iso-propanol. Extracted samples were reconstituted in 45/55 methanol/ammonium acetate buffer, pH 4.0 and injected (10 uL) onto a phenyl-hexyl column using gradient elution with methanol and a flow rate of 0.3 mL/min. The complete run time with column reconditioning was 13 min. Buprenorphine-D4 was used as internal standard. LC/MSMS system consisting of Shimadzu LC-lOAdvp pumps, SIL-HTC autosampler and Sciex API300 triple quad MSMS (Ionics EP10+ upgrade).

The method extraction efficiency was above 80% for all drugs except pseudoephedrine (66%). In order to minimize matrix effect and expand linearity range, the extracted sample was diluted with reconstitution solution to its initial extraction volume. This "sacrifice" in method sensitivity resulted the LOQ of 10 ng/mL and above for most of the tested drugs. The obtained LOQ were well below 40% of their individual cut-off levels including the lowest LOQ of 0.075 ng/mL achieved for fentanyl.

This study has demonstrated that LC/MSMS technology could be employed for urine multi-drug screening. This method is flexible and sensitive enough to replace several analytical techniques previously used for the same task.

Key Words: LCMS, forensic drugs, screening

P9

Positive Pressure Solid Phase Extraction and GC/MS Analysis of 6-Monoacetylmorphine in Urine for Production Forensic Drug Testing Labs

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An improved method for the detection of 6-monoacetylmorphine in urine by gas chromatography-mass spectrometry is presented. The improved method meets present requirements for both the Department of Defense and the National Laboratory Certification Program (NLCP). The method uses a 3mL sample size, eliminates keto-opiates using sodium bisulfite, is linear from 4-250 ng/mL using a single-point calibration, has an limit of detection (LOD) of 3 ng/mL and has a retention time of approximately 6 minutes.

Extraction uses a positive pressure manifold fitted with SPEware Cerex Polycrom Clinll solid phase extraction cartridges. Samples are derivatized using BSTFA and GC/MS analysis is performed on an Agilent Technologies 6890GC/5973N MSD using a J&W Column 1 Om, 0.18mm id, 0.1 Sum film thickness.

Side by side comparison with the ANSYS SPEC columns and derivatization with BSTFA shows improvement using SPEware and Cerex Polycrom Clin II extraction cartridges. Using the parent +1 ion of 399 amu and daughter ions of 287 and 340 amu, the method is able to meet and exceed the Department of Defense LOD and LOQ requirements and meet the NLCP requirement of an LOD (3 ng/mL) and LOQ (4 ng/mL). Previously it was necessary to monitor the 399, 400 and 340 ions in order to meet NLCP LOD requirements. This poses a concern since the 399 and 400 ions are the same compound/fragment demonstrating a silicon isotope affect of the BSTFA derivative.

The improved method is a clean and rugged extraction from a urine matrix. The method reduces the amount of sample required for extraction from *5* mL to 3 mL. Using SPEware positive pressure manifolds cuts the extraction time in half. The method is also able to meet both the NLCP and Department of Defense requirements for LOD while extending the upper limit of linearity.

Key Words: 6-monoacetylmorphine (6-MAM), solid phase extraction (SPE), gas chromatography-mass spectrometry (GC/MS)

P10

ELISA Screen for Human Insulin and Insulin Analogs in Serum

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Insulin is the major hormone used by the body to regulate glucose metabolism. It begins as proinsulin existing as two connected segments - insulin and C-Peptide. When insulin is in demand, proinsulin is cleaved, providing insulin and C-Peptide to the bloodstream in equimolar amounts. Insulin is metabolically active and taken up by tissues to metabolize glucose. In contrast, C-Peptide is not metabolically active.

People who suffer from insulin-dependant diabetes (Type 1) cannot produce proinsulin, so insulin (or insulin analog) must be administered. If too much insulin is administered, or if it is administered to a non-diabetic, it can be fatal. Of the analogs available as injectable solutions, some are identical in structure to human insulin, some differ only in the arrangement of the amino acid sequence, and yet others have additional amino acids added to their sequence. Currently, few forensic laboratories have the capability to screen biological specimens for insulin or insulin analogs. Even in a clinical setting, it is typical that glucose levels are monitored instead of insulin levels when diagnosing and treating diabetes.

There were two objectives for this study. The first was to determine the cross-reactivity of a series of insulin analogs with three commercially available ELISA kits, each targeting different insulin-related components. The second was to examine the effectiveness of these three ELISA kits to determine exogenous insulin administration.

The three ELISA kits will be referred to as Insulin, Isolnsulin, and C-Peptide. The Insulin kit is designed to detect human insulin, and any analogs identical in structure to human insulin. The Isolnsulin kit is designed to detect human insulin as well as insulin analogs. The C-Peptide is designed to just detect C-Peptide. The insulin analogs studied include Regular Human Insulin, Insulin Aspart, Insulin Glargine, Lente, and Lispro.

A validation study was performed to determine the selectivity, precision, and limit of detection of all three ELISA kits. Human serum was spiked with the above mentioned analogs for concentrations of 30 and 100 uU/mL above the endogenous insulin level. In addition, human serum samples were obtained from 4 volunteers, one of which was an insulin-dependant diabetic. The volunteer samples were collected at various times during the day so as to provide a fasting sample, a sample immediately following a meal, and a sample a few hours after a meal. All samples were analyzed using the three different ELISA kits.

Cross-reactivity studies of the five analogs are presented, as well as the results of the four human serum samples. Finally, a suggestion for the interpretation of the collective data is offered.

Key Words: Insulin, Insulin Analogs, ELISA

P11

Validation of Commercial ELISA Immunoassay Kits beyond Manufacturer Specifications

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Enzyme-linked immunosorbent assays (ELISA) are used in many forensic laboratories to screen biological fluids for the presence of drugs of abuse. The technique is rapid and simple to perform, and kits for the analysis of a wide variety of drugs are available from numerous commercial sources. Unfortunately, the cutoff levels specified "out of the box" for many of these kits are fairly high, since the manufacturers tend to target the emergency toxicology, driving under the influence, and workplace drug testing markets. This can present problems for laboratories wishing to broadly screen submitted samples in cases where low drug levels are of concern, including the investigation of drug-facilitated sexual assault and certain public corruption cases. Also, most commercial ELISA kits are designed for urine or serum analysis, but whole blood is the primary specimen for many forensic toxicology investigations, and is sometimes the only available liquid specimen.

We present the protocols used in our laboratory for revalidation of commercial ELISA kits for use on whole blood and urine at cutoff levels below manufacturers' default specifications. Appropriate matrix calibrators are prepared at two levels for each target compound: the proposed cutoff, and a positive at 4-5 times the cutoff concentration. These samples, along with negative matrix from at least four different sources, are assayed multiple times over several days, and the resulting combined data are evaluated for separation between the three levels. The results are considered acceptable for validation if the negative data are separated from the cutoff data with at least 10,000:1 confidence, and the cutoff and positive data are separated with at least 1,000:1 confidence. Data from the successful validation of kits from three different manufacturers, including amphetamine, barbiturates, benzodiazepines, cannabinoids, cansoprodol, cocaine metabolite, ketamine, LSD, methadone, methamphetamine, opiates, PCP, tricyclic antidepressants, and zolpidem are presented.

KeyWords: ELISA, Immunoassay, Validation

P12

Long-term Storage Stability of Topiramate

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Topiramate is a relatively new antiepileptic drug that is indicated for the adjunctive treatment of a variety of seizure syndromes in adults and children 2 years of age and older. It is derived from the D-enantiomer of fructose and is classified structurally as a sulfamate-substituted monosaccharide (2,3:4,5-bis-O-(l-methylethylidene)-β-D-fructopyranose sulfamate). Topiramate was thought to possess a wide margin of safety but reports of metabolic acidosis required the modification of package inserts to highlight this potentially serious adverse effect. There have been two reports of fatalities attributed to topiramate intoxication. Additionally, patients with epilepsy have a high risk of sudden death, and noncompliance with therapeutic regimens may be a predisposing factor. Degradation of topiramate in storage could lead to false interpretations due to potentially fatal and therapeutic concentrations at the time of death appearing to be therapeutic and noncompliance, respectively, at the time of analysis. Therefore, because of the increased forensic relevance for determining the presence of topiramate in death investigations, the long-term storage stability of topiramate in preserved blood was assessed.

Blood donated from the Canadian Blood Service was allowed to stand at 20 - 22°C (room temperature) for approximately one month. Afterwards, sodium fluoride (1%) and sodium citrate (0.5%) were added as preservative and anticoagulant, respectively. The blood was then spiked with a sufficient volume of a topiramate stock solution to produce target concentrations of 20 and 100 mg/L. Aliquots (10 mL) of spiked blood were stored in 16 mL polystyrene screw-cap Falcon® tubes at room temperature, 4°C, and -80°C. Analyses to determine topiramate concentration were conducted following 0, 1, 2, 3, 6, 9, 12, and 24 months of storage.

Topiramate was extracted by precipitating 250 µL of blood with 3 mL of 9:1 MeOH:H_2O. A portion (2 µL) of the resulting supernatant was injected into a TSQ Quantum LC-ESI-MS. TSQ Quantum conditions, i.e., collision energy and tube lens voltages, were optimized in ESI/MS positive polarity mode. Analyses were performed in Selected Reaction Monitoring mode. Standard curves were constructed with the following concentrations: 6.25, 12.5, 25, 50, and 100 mg/L, with droperidol (20 mg/L) as internal standard. Two daughter ions were monitored for topiramate (m/z 264, 340) and for droperidol (m/z 165, 194). Aged preserved blood to which topiramate had not been added (blank) and a single-blind (quality control) sample was included with each analysis.

The initial analysis (Day 0) produced results (mean ± SE) of 94.3 ± 2.9% and 93.0 ± 2.9% of the 20 and 100 mg/L target concentrations, respectively. For each analysis, five replicate analyses were conducted on samples from each concentration x storage temperature condition. The within %CV was < 10% for all analyses. When compared with the results obtained on Day 0, topiramate was observed to be remarkably stable in preserved blood regardless of concentration or storage temperature. The overall mean (± SE) percent (and range) of the 20 mg/L target concentration at room temperature, 4°C, and -80°C were 98.7 ± 3.5% (85.7 - 110.8%), 92.9 ± 2.1% (84.0 - 99.3%), and 93.7 ± 1.7% (85.9 - 99.5%). Similarly, the respective overall means for the 100 mg/L target concentration were 97.9 ± 2.4% (87.1 - 103.6%), 88.1 ± 2.0% (78.9 - 95.6%), and 87.8 ± 1.8% (82.9 - 94.2%). Degradation of torpiramate in preserved blood does not appear to be a significant forensic issue.

Key Words: Topiramate, LC/MS/MS, stability

P13

Ethanol Analysis From Biological Samples By Dual Arm Robotic Autosampler

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Detection, identification, and quantitation of ethanol and other low molecular weight volatile compounds in liquid matrices by automated headspace gas chromatography-flame ionization detection (GC-FID) is common practice in most forensic laboratories. Although it is one of the most frequently utilized procedures, sample preparation is still usually done manually. Instituting the use of a dual-armed programmable autosampler can eliminate the need for sample preparation altogether.

A CTC dual-armed programmable autosampler (PAL) is configured so that one arm is used for sample preparation and the other is used as a traditional autosampler arm. The sample preparation arm draws up and sequentially adds brine (saturated sodium chloride) solution, de-ionized water, and internal standard (acetonitrile) to a headspace vial containing a biological sample, a calibrator, or a control. After sample preparation is completed, the autosampler arm moves the sample to the agitator for incubation, and then positions it for sampling and injecting the headspace.

Several sample sets were analyzed, each consisting of: established ethanol calibrators (0.025%, 0.05%, 0.080%, 0.100%, 0.200%, 0.300% w/v), a negative control, and bi-level positive controls (ethanol, methanol, isopropanol, acetone, and acetaldehyde). All peaks demonstrated good chromatographic fidelity (reasonable peak shape, width, and resolution). Limits of detection, lower limit of quantitation, and linearity were comparable to the current manually prepared method, while maintaining acceptable accuracy and precision.

Key Words: ethanol, robotics, autosampler

P14

Acute Poisoning with Brake Fluid

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Abstract:

This paper presents the case of a 29-year-old male who was found in his apartment by Miami-Dade Fire Rescue and subsequently transported to a local hospital where he later expired. The victim had telephoned a friend and advised him that he had swallowed brake fluid in an attempt to commit suicide due to domestic problems. At the time of autopsy the only remarkable finding was the smell of industrial solvents emitting from the deceased's body. Postmortem fluids and tissues were sent to toxicology for further analyses. Brake fluid components was identified and confirmed by full scan electron ionization gas chromatography-mass spectrometry in the following postmortem specimens: blood, urine, brain, liver, kidney, and gastric lavage. A simple liquid-liquid extraction procedure was used to isolate the key components of brake fluid. The major components of brake fluid are 2-[2-(2-ethoxyethoxy) ethoxy]-ethanol and l-[(2-butoxyethoxy)]-ethanol. The primary ions used for identification were: 45 (base peak), 59 (major ion), and 89 (major ion). Due to the toxicology findings and circumstances surrounding the decedent's death, the medical examiner ruled that the pending cause of death was acute poisoning with brake fluid.

Key Words: brake fluid, poisoning, suicide

P15

Use of GC and Tandem MS/MS for Confirmation and Quantitation of Amphetamines and other Phenethylamines in Oral Fluid

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Purpose: To describe a method for the analysis of amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), and methylenedioxyethylaniphetamine (MDEA) in oral fluids using gas chromatography and ion trap tandem mass spectrometry (GC-MS/MS).

Use of oral fluids as a matrix for drugs of abuse testing has gained increased acceptance, and in 2004, the United States Substance Abuse Mental Health Services Administration (SAMHSA) proposed revisions to the mandatory guidelines for federal workplace drug testing programs that would allow oral fluid testing for federal employees (1). Confirmation of drug use in oral fluids following an initial screening test can be performed by gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), GC-tandem MS (triple quadrupole or ion trap) or LC-tandem MS (triple quadrupole or ion trap). Because of the nature of the matrix and the low concentrations of target compounds in oral fluids, a GC-MS/MS method using an ion trap was developed for the analysis of AM, MA, MDA, MDMA and MDEA in oral fluids. This method was then validated according to SAMHSA method validation guidelines, including range of linearity; limits of detection and quantitation; precision at the cutoff and at 40% of the cutoff, and potential for interference (1).

An MS/MS method was developed that optimized precursor ion production by comparing both electron impact and chemical ionization approaches, and which optimized product ion production so as to provide multiple ions for quantitation and ion ratio confirmation. This process was conducted for each target analyte and its associated deuterated analog, which acted as an internal standard. The gas chromatographic method allowed full separation of target compounds to allow for optimal isolation of targets and internal standards. Following method development, the method linear range extended from 2.0 ng/mL to 500 ng/mL for all components. At the proposed cutoff of 50 ng/mL for each target compound and a 100 uL sample size, the limit of detection for the assay was 5 ng/mL for AM, MA and MDEA, and it was 2 ng/mL for both MDA and MDMA. Precision at the cutoff and at 40% of the cutoff was within acceptable limits (<10% Coefficient of Variation, n = 7). A number of compounds were assessed for potential interference in the assay, and it was found to be free of interference, demonstrating high specificity.

Key Words: Oral Fluids, GC-MS/MS, phenethylamines

P16

A Retrospective Review of Methadone Deaths: A Toxicology Study

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Methadone is in many countries the treatment of choice in opiate addiction. In spite of its widespread use, methadone is not as safer as it would be desirable and deaths are frequently reported in relation to it. The present paper studies methadone and its main metabolite distribution in 254 cases of fatalities in which several kinds of specimens are analysed (blood, urine, gastric contents, bile and vitreous humour). In an attempt of helping to determine whether a post-mortem concentration should be attributed to either therapeutic ingestion or overdose, we have calculated EDDP/methadone concentration mean relationship for every type of specimen analysed. Also we compare urine concentrations of both compounds in fatalities with urine concentrations in several cases of living methadone consumers. EDDP/methadone concentration mean relationship was also calculated and compared between the two groups looking for significant differences. All cases presented here were analysed in the Department of Seville of Spanish National Institute of Toxicology from June of 2002 to the first semester of 2004.

Being the aim of this paper, to give more data on methadone behaviour in the body and if possible improve the interpretation of methadone post-mortem concentrations, we can state:

1. Polydrug use is widespread in Southern Spain
2. Only in a low percentage of deaths (18'25%) can be related directly to a high blood methadone concentration, being in most of the cases within the therapeutic interval.
3. Information in revised literature on methadone or main metabolite concentrations in specimens different from blood is sparse.
4. EDDP/Methadone urine ratios are different in living addicts than in deceased people ($p<0'05$).
5. It would be advisable to perform a comprehensive toxicological analysis, which includes stomach contents, bile and vitreous humour, to improve interpretation.

Key Words: post-mortem, distribution, EDDP/methadone

P17

Distribution Characteristics of Opiates in Urine and Hair Specimens Collected from Alleged Heroin-Users in Northern Taiwan

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This study was conducted to better understand the distribution characteristics of 6-acetylmorphine (6-Am) and free and total morphine and codeine (F-Mor, F-Cod, T-Mor, T-Cod) in urine and hair specimens. It is anticipated that information hereby gathered can help design a more effective test strategy for the identification of heroin users.

Twenty-six alleged heroin-using arrestees from Keelung Police Department (north of Taipei, Taiwan) consented to participate and contributed 8 sets of urine-hair paired, 14 sets of urine, and 4 sets of hair specimens. Each set of urine included seven specimens collected at the following hours after the arrest: 0, 12, 24, 48, 72, 96, and 120. Hair specimens were cut into 2-cm sections. All urine and hair specimens were analyzed without and with hydrolysis to determine the concentrations of 6-Am, F-Mor, F-Cod, T-Mor, and T-Cod. The detection limits of the urine protocols were 1 ng/mL for 6-Am and 10 ng/mL for the other 4 compounds, while the detection limits for the hair protocols for all compounds were 0.2 ng/mg. =

Among the 8 urine-hair paired sets, 6-Am was detected in 7 urine specimens collected at time "0" and in the first sections (0-2 cm from the root) of 7 sets of hair specimens. The urine specimen without detectable 6-AM was not collected from the same subject without detectable 6-Am in the hair.

6-Am was not detected in all 22 (14 + 8) urine specimens collected at 12 hours after the arrest, even the one with the highest 6-Am concentration (636 ng/mL) at time "0". The concentration ranges of T-Mor and F-Mor for the urine specimens collected at time "0" were 215 to 11400 ng/mL and not detectable to 7110 ng/mL.

Among the 12(8 + 4) sets of hair specimens, 6-AM, F-Cod, and F-Mor were not detected in the 0-2 cm sections of 2, 8, and 7 sets of specimens, respectively. None of the monitored compounds was detected in the hair specimen collected from 1 subject who did not provide urine specimen.

In conclusion, the analysis of both hair and urine specimens collected at the time of arrest can provide complementary information to determine whether the subject of concerned has had heroin exposure.

Key Words: Heroin, Urine, Hair

P18

Donepezil (Aricept®): An Incidental Finding in Eleven Postmortem Cases

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Donepezil (Aricept®) is a reversible inhibitor of acetylcholinesterase. The Food and Drug Administration (PDA) approved the drug in December 1996 to treat patients with mild to moderate dementia associated with Alzheimer's disease. Donepezil is supplied in three forms: 5 and 10 mg tablets, 5 and 10 mg disintegrating tablets, and 1 mg/mL solutions, with typical doses not to exceed 10 mg per day. Therapeutic concentrations have been reported to range from 0.003-0.031 mg/L. The Los Angeles County Department of Coroner Toxicology Laboratory first detected Donepezil during a postmortem investigation in September 2003 and has since encountered ten more cases where Donepezil was considered an incidental finding. The objective of this paper is to provide general information on Donepezil, including postmortem concentrations, which will aid the toxicologist in the interpretation of casework.

Postmortem specimens from eleven cases were analyzed utilizing a basic liquid/liquid extraction for the presence of Donepezil. Screening and quantitation was performed on a gas chromatograph nitrogen phosphorous detector (GC/NPD), while confirmation was determined on a gas chromatograph mass spectrometer (GC/MS). Linearity was achieved from 0.10 to 5.0 mg/L, with a limit of quantitation of 0.10 mg/L. The tissue distribution range of Donepezil in the eleven cases were as follows: central blood +<0.10-0.38 mg/L (5 cases), femoral blood +<0.10-0.29 mg/L (7), liver 4.0-5.0 mg/kg (2), gastric contents 0.10 mg total (1), vitreous +<0.10-0.35 mg/L (3), bile 0.72 mg/L (1) and urine +O.10-0.65 mg/L (4). Case histories, autopsy findings, other toxicology findings, and the cause and manner of death for each of the eleven cases are presented. It is the author's belief that this is one of the first reports documenting Donepezil concentrations in postmortem specimens.

KeyWords: Donepezil, Postmortem, Tissue Distribution

P19

Study of Oxycodone Occurrences at a Large City Medical Examiners' Office

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Introduction: Recent reports in the news media have indicated that use of street drugs such as Ecstasy, which are usually obtained from sources outside the United States, have declined because of increased homeland security post 9/11. It is postulated that prescription drugs such as oxycodone are being used as "safer" replacements. In addition, other reports show that because of the heightened awareness of abuse of the extended release form of oxycodone (OxyContin™), the number of legal prescriptions of the drug may be decreasing. This study from a large city MEO was undertaken to determine the frequency of oxycodone as the cause of death in 2003 and 2004, either singly or in combination with other drugs.

Methods: Post-mortem data was obtained from a computer database (CME) for all deaths involving oxycodone for the years 2003 and 2004. Other drugs/drug classes that were detected concurrently with the oxycodone were broken down into the following groups: cocaine and metabolites, ethanol, antidepressants/antipsychotics , narcotics, PCP and benzodiazepines. All drug screen results were from fluoridated blood or urine and were confirmed by GC-MS except for benzodiazepines where indicated. No attempt was made to discriminate between the presence of any one specific product of the Schedule II oxycodone containing products available in the United States.

Results: Oxycodone: In 2003 the Toxicology laboratory at the Philadelphia Medical Examiners' Office tested specimens from 2094 cases. Eighty- two (3.9.%) of these cases were positive for oxycodone. In fifty- four (65.6%) of the cases positive for oxycodone, the cause of death was determined to be either drug induced or drug related. In 2004, specimens were tested from 2067 cases. One hundred two (4.9%)of these cases were positive for oxycodone. In sixty- three (61.8%) of the positive oxycodone cases, the cause of death was determined to be either drug induced or drug related. Concurrence of the other drugs/drug classes in drug abuse cases followed a similar pattern in both years with benzodiazepines > narcotics > antidepressants/antipsychotics > cocaine and metabolites > ethanol > PCP. Other abused drugs: As a comparison, in 2003 cocaine, the most commonly seen drug of abuse seen at our MEO, was positive in 302 cases (14.4 % of total cases) with 171 (56.7%) of these deaths attributed to drug abuse. Similar statistics were seen in 2004: 357 positive cocaine cases (17.2% of total cases) with 209 (58.5%) of these deaths attributed to drug abuse.

Conclusions: In our two-year study, no drug abuse cases (total = 107) were found where oxycodone was the sole drug present. A high prevalence of concurrent use of benzodiazepines, antidepressants/antipsychotics, narcotics and cocaine as well as occasional use of ethanol and PCP was seen. In lieu of the fact that the percentage of cases of oxycodone determined to be drug abuse cases actually increased slightly to 3.0% (63/2067) in 2004 from 2.6% (54/2094) in 2003, it is clear that oxycodone continues to be a very common drug found in overdose cases and that its role should be recognized as significant in these deaths. Consequently, special care needs to be taken to ensure that toxicology testing detects oxycodone. In many cases this requires GC/MS since cross-reactivity to oxycodone in most immunoassays for the opiate class is very low. In addition, data from our laboratory indicates that both Ecstasy and methamphetamine are infrequently seen as causes of death in Philadelphia.

Key Words: Oxycodone, Postmortem toxicology, Drugs of Abuse

P20

Zolpidem as a Primary Cause of Death- Case Report

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We wish to report on a fatal intoxication involving Zolpidem as the primary agent. Zolpidem is an imidazopyridine hypnotic that binds to the GABA A receptor. It's primary use is in the short-term management of insomnia, but it has also been reported to be effective in other conditions such as blepharospasm, spinocerebellar ataxia, and in jet lag. Zolpidem has an excellent safety record since its introduction in 1988, with positive reports in the elderly also being reported. Expected side effects such as psychomotor function impairment and memory impairment are reported, but it fairs well compared to other benzodiazepines. Other side effects such as distortion of visual perception and epileptic seizures have been reported at therapeutic doses. Acute non-lethal intoxication is also reported with the largest series containing 344 cases of intentional overdose. Many patients ingest psychotropics and alcohol concurrently. Zolpidem has also been used in drug facilitated crimes including sexual assault. Fatalities involving Zolpidem have been reported, but the occurrence of Zolpidem overdose as the primary cause of death is rare.

We wish to report on an 80-year-old man who was found having locked himself in his flat. There was a packet containing Zolpidem (lOmg) tablets, of which fourteen could not be accounted for. Alongside this were an empty, half litre brandy bottle, Seretide and Ventolin inhaler, Temazepam tablets and an empty container with the label Lorazepam. The individual was also known to be on the following medication: Furosemide, Clopidegril, Ramapril, Isosorbide mononitrate, Seretide, GTN spray, Coproxamol, Prochloperazine, Simvastatin and Tildiem retard.

The post mortem revealed some features of pre-existing ischaemic heart disease and chronic obstructive pulmonary disease, but with no evidence of any acute events to explain death.

Toxicological investigation revealed a blood Zolpidem concentration of 6.86mg/L. Toxicity is reportedly associated with levels above 500ng/L. The stomach-content concentration was 35.1 mg/L. Zolpidem was detected and quantified using GCMS. Blood alcohol was 93mg/100mL. Other drugs detected in blood were Paracetamol <5mg/L, Detropropoxyphene 0.45mg/L, Tramadol 0.89mg/L and Diltiazem 0.33mg/L, all of which may reflect therapeutic range use.

The perception that Zolipdem is a relatively safe drug has lead to an increase in its prescription. This is likely to lead to it becoming more prevalent both as a primary toxin and in combination with other drugs used in overdose.

Key Words: Zolpidem, benzodiazepine, overdose

P21

Therapeutic and Toxic Concentrations of Mirtazapine (Remeron®) in Postmortem Cases

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Mirtazapine (Remeron®-US, Zispin®-UK) is a tetracyclic piperazinoazepine compound indicated for the treatment of major depression in the United Stated since 1996. Mirtazapine has been described as a noradrenergic and specific serotonergic antidepressant (NaSSa). Unlike SSRIs that are commonly being prescribed for depression, mirtazapine does not broadly block the re-uptake of serotonin. Instead, it stimulates the release of both norepinephrine and serotonin while blocking two of the serotonin receptors thought to be responsible for many of the undesirable side effects of traditional SSRIs. Clinical trials of mirtazapine indicate an excellent safety profile with the major side effect being somnolence. Remeron® is available in 15 mg, 30 mg and 45 mg tablets, taken once daily.

Mirtazapine is among the new antidepressants being widely prescribed. In a study among primary physicians, newer non-SSRIs comprised 17% of all antidepressants prescribed, while older tricyclic antidepressants were prescribed at a rate of 1 %. As more of these non-SSRIs enter the market, it has become important to be able to equate therapeutic levels with potentially toxic levels.

Six cases involving mirtazapine were analyzed at the San Diego County Medical Examiner's Office from 2004-2005. Mirtazapine was detected in each of these cases by a liquid-liquid GC/MS basic drug screen. Following a liquid-liquid basic extraction, mirtazapine was confirmed and quantitated by gas chromatography with nitrogen phosphorous detection. For each case, mirtazapine levels in peripheral blood (pb), central blood (cb), vitreous (vit) and liver were determined against matrix specific calibration curves (LOD 0.01 mg/L, LOQ 0.025-1.0 mg/L). In contrast with earlier studies of postmortem distribution of mirtazapine, we found concentrations in liver that were significantly higher. Mirtazapine was implicated in the cause of death in three of these six cases. In these drug related deaths, the mirtazapine concentrations were 2.0 ± 1.5 mg/L (pb), 1.6 ± 1.0 mg/L (cb), 0.78 ± 0.56 mg/L (vit) and 10 ± 7.4 mg/kg (liver). Alternatively, concentrations considered therapeutic (three drug unrelated deaths) were 0.18 ± 0.22 mg/L (pb), 0.16 ± 0.17 mg/L (cb), 0.12 ± 0.16 mg/L (vit) and 0.73 ± 0.68 mg/kg (liver). While mirtazapine levels were elevated in blood and liver in three cases, it should be noted that other drugs were also found in toxic concentrations in each case. This may further support the fact that mirtazapine is a safe drug with respect to overdose.

P22

Tissue Distribution of Loperamide and N-Desmethylloperamide Following a Fatal Overdose

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We report a case involving a fatal intoxication with loperamide. A 26-year old male was found unresponsive, face down on the side of the road approximately 3 hours after last being seen. Superficial abrasions to the head were noted but no other injury was seen at autopsy. The subjects' medications at the time of death included lithium carbonate, trazodone, and aripiprazole, with remaining doses consistent with proper use. The decedent had reportedly been taking OTC anti-diarrheal medication as a frequent self-treatment for "stomach problems", and a generic version of loperamide was found with the body. Loperamide is a synthetic opioid of the phenyl piperidine class used as an over-the-counter antidiarrheal. It exerts its effects through interaction with D -opiate receptors in the intestine to reduce peristalsis. Loperamide lacks the typical euphoric opiate effects when administered at recommended doses. Both loperamide and its major metabolite, N-desmethylloperamide, were isolated by liquid-liquid extraction into n-butyl chloride from alkalinized samples. Extracts were analyzed by liquid chromatography / electrospray-mass spectrometry in selected-ion-monitoring mode. Rapid separation of the drug, metabolite, and internal standard (diphenoxylate) was achieved using a high resolution CIS column with 1.8 Dm particle diameter. The mobile phase consisted of 0.1% formic acid in deionized water (60%) and acetonitrile (40%) at a flow rate of 0.5 mL/min. The positive ions of loperamide *(m/z* 477, 266, 210), N-desmethylloperamide *(m/z* 463, 252, 196), and diphenoxylate *(m/z* 453, 425) were formed by electrospray ionization. Quantitative values are listed in Table 1. Heart blood concentrations for loperamide and its metabolite were 1.2 mg/L and 3.3 mg/L, respectively. In contrast, reported peak plasma concentrations of loperamide after administration of recommended daily doses of 16mg did not exceed 0.012 mg/L in controlled trials. Since the heart blood ethanol concentration was 0.08 g/dL, the medical examiner ruled that the cause of death was loperamide and ethanol intoxication and the manner of death as undetermined.

Table 1: Tissue distribution of loperamide and N-desmethylloperamide

	Central Blood mg/L	Peripheral Blood mg/L	Gastric mg	Bile mg/L	Kidney mg/kg	Liver mg/kg	Urine mg/L
Loperamide	**1.2**	**2.6**	**3.9**	**8.9**	**8.5**	**12.5**	**9.2**
N-desmethyl loperamide	**3.3**	**6.4**	**1.2**	**44.6**	**31.7**	**30.6**	**44**

Key Words: Loperamide, Overdose, Postmortem

P23

Dynamic Headspace Extraction as an Alternative to Solid Phase Microextraction for the Analysis of Volatile Organic Compounds at Physiological Levels in Blood

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Solid phase microextraction (SPME) has recently become a technique of choice for the analysis of volatile organic compounds in difficult biological matrices. Lately, however, a robust method of automated volatile sample preparation for GC/MS analysis was made commercially available, at a cost competitive with the large initial expenses necessary for the instrumental setup of a robotic SPME apparatus. In comparison to SPME, dynamic trapped headspace technologies replace the trapping of volatile organic compounds (VOCs) on a fiber-based film of adsorbent with a trap similar to those used in purge-and-trap headspace for environmental analytical methods. This instrumental setup not only allows for a direct transfer of analytes concentrated over time from the headspace of the sample into the GC inlet via an airtight and chemically inert line, but also maintains the analytes of interest in a concentrated "band" during the process of moving between two machines. In relation to analytical work in blood, dynamic headspace appears to offer the advantage of VOC separation without the requirement of cryogenic cooling, giving an increased cost-savings and a lower level of technical expertise required for instrumental operation. Our experiences with dynamic headspace equipment indicate that the parts-per-trillion (ng/L) level detection limits obtained by others, a necessary step in determining physiological levels of these compounds in clinical specimens, are achievable at a reduced cost, and that comparable data quality can be realized.

Key Words: Volatile, Organics, Blood

P24

Analysis of Coniine in Blood and Gastric in a Poison Hemlock Death

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A 27-year old female, with a history of bipolar disorder and previous suicide attempts, was found deceased inside of a sleeping bag in a relatively isolated wooded area of northern California. A plastic ziplock bag containing a green herbal leafy material was found situated in very close proximity to the body along with some personal belongings. While there was no evidence of weapons, illicit drugs or alcohol at the immediate location of the deceased, her car contained a variety of vitamins in addition to risperidone and ziprasidone tablets. At autopsy it was seen that the green leafy material was inside of her mouth and present in her gastric contents as well. In addition, pulmonary and cerebral edemas were observed. Peripheral blood and gastric contents were submitted to National Medical Services from Santa Clara County Coroner for toxicological analysis including a request to help determine if the deceased could be a victim of hemlock poisoning. At the time of specimen receipt it was observed and documented that the gastric content was rich with bits of a leafy material and green in color, as if the components that give the plant its color had diffused from the plant itself into the gastric fluid. Poison Hemlock (Conium Maculatum) is a biennial member of the carrot family that grows wild throughout the US especially along roadsides. Socrates is thought to have died from ingestion of Poison Hemlock. The toxicity of Poison Hemlock is from several simple piperidine alkaloids including coniine, gamma-coniceine, conhydrine, N-methylconiine, and pseudoconhydrine. Coniine and gamma-coniceine are thought to have the most significant contribution to the toxic affects, which are similar to nicotine poisoning.

Because a reference standard was available for coniine and as coniine is considered to be one of the more important hemlock constituents, our bioanalytical testing focused on the identification and quantitation of this compound in blood and gastric by full scan and SIM GC/MS. In addition, we also attempted to confirm the presence of the other alkaloids by full scan GC/MS. Analysis was performed by a solid phase extraction followed by reaction with butyric anhydride and GC/MS-SIM analysis using methylnicotine as the internal standard. The method provided a linear response with acceptable ion ratios over a concentration range of 20 to 1000 ng/mL. The blood and gastric specimens were analyzed on multiple dilutions and tested with 2 levels of standard addition to show specimen specific recovery. Based upon the results of standard additions the coniine concentration in the blood was 410 ng/mL while its concentration in the gastric was 9300 ng/mL. Several other piperidine alkaloids were tentatively identified based on their mass spectra in both the blood and gastric specimens.

Comprehensive toxicology screening and confirmation tests were performed. Findings from this testing on the blood specimen include diazepam at 81 ng/mL, nordiazepam present at less than 50 ng/mL. Ziprasidone and risperidone were not found in the blood by LC/MS/MS. To our knowledge, this is one of the few cases of death by hemlock where the concentrations of coniine were determined. Because the lethal dose of hemlock poisoning is unknown in humans, this report begins to help verify what this lethal concentration may be. In the absence of a more competent cause, we suggest that this death can be solely attributed to hemlock poisoning.

Key Words: Poison Hemlock, coniine in blood and gastric, and post-mortem

P25

Blood and Liver Olanzapine Findings in Fourteen Postmortem Cases

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Objective: To evaluate toxicology findings of the neuroleptic agent olanzapine in a series of deaths to assist in the interpretation of postmortem olanzapine findings.

Methods: A retrospective study was conducted of the years 2003 and 2004 of the toxicology laboratory of the Office of the Chief Medical Examiner of North Carolina for olanzapine positive cases. Demographics of the decedents, olanzapine findings, and the cause and manner of death as ruled by the medical examiner were noted. Olanzapine was initially detected in blood by alkaline liquid/liquid extraction with n-butyl chloride/ether mixture. Extracts were back-extracted into acid, extracted with hexane for cleanup and following sample alkalization isolated with butyl chloride. The residues were then analyzed by GC/MS in a DB-5MS column (15m x 0.25mm id x 25 *um* film thickness) at the following temperatures: initial, 70°C; ramp, 15 °C/min; finial 250 °C; yielding retention times: olanzapine, 15.1 min. and alphaprodine (IS), 8.73 min. Olanzapine ions monitored were 242/229/312 m/z. Olanzapine was also detected in acid fraction GC/MS blood drug screens. Quantification of olanzapine in blood and liver homogenates (1:4 dilution) involved isolation by solid phase extraction in United Chemical Technologies Clean Screen columns (CSDAU206). Olanzapine was converted to its trifiuoroacetyl (TFAA) derivative and chromatographed on the column described above at the following temperatures: initial, 120°C; ramp, 30°C/min; finial 300 °C. Olanzapine-TFAA yielded ions of 338/309/323. Typical calibrations were from 0.20 - 4.0mg/L with clonapine as the internal standard applying quantification at ions 338/191.

Results: Olanzapine was detected in 14 deaths over the two years of this study. Two were fatal intoxications solely due to olanzapine; both were women, 46 and 47 years old, with blood olanzapine values of 0.81 and 1.5 mg/L, respectively. Twelve deaths involved multiple drug overdoses of 8 women, ages 35 to 68 years and 4 men, aged 17 to 73 years. Olanzapine findings were; mean aorta blood, 1.57 mg/L (range, 0.11 to 4.5 mg/L, N=12) and mean liver, 9.8 mg/Kg (range, 0.74 to 27 mg/Kg, N = 10). Antidepressants, anxiolytics and analgesic drugs were also present in nine of these multiple drug deaths and ethanol contributed to another. Blood values were generally higher than those previously reported in olanzapine overdose fatalities. Few liver olanzapine concentrations have been previously reported. Iliac blood specimens were also submitted in 7 of the 14 total cases. Aorta blood olanzapine concentrations were on average 120% higher (range 40 to 300%) than iliac blood values.

Conclusion: From these data, olanzapine aorta blood concentrations of 0.8 mg/L or greater or iliac blood values of 0.5 mg/L or greater are sufficient to cause death. In instances of olanzapine overdose, liver concentrations can be expected to exceed 1.0 mg/Kg. Olanzapine demonstrates site dependent blood concentrations.

Key Words: Olanzapine, Drug Overdose, Solid Phase Extraction, GC/MS

P26

Paraquat in Exhumed Remains by Gas Chromaitography-Mass Spectrometry

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Paraquat is a bis-quaternary ammonium compound that has been used as an herbicide since the 1960's. Due to its toxicity in humans, paraquat's use has been restricted in the United States. Exposure to paraquat can cause damage to the gastrointestinal lining, as well as distribute to other tissues such as the liver, kidney and lungs. This case report highlights a simple method that utilizes a liquid-liquid extraction procedure followed by gas chromatography-mass spectrometry analysis for the detection of a reduction product of paraquat in various biological tissues. The validated method was applied to fluids and tissues obtained from exhumed remains.

The decedent was a 75-year-old man applying paraquat herbicide around his orange groves when the hand-pumped pressurized canister exploded, spraying the liquid onto his lower extremities. He showered at home and then presented to the ER about 4 hours later complaining of nausea, shortness of breath, increased salivation and vomiting. His past medical history was significant for coronary artery disease, nephrolithiasis and alcoholic cirrhosis. In the ER, he developed anterior chest pain and laboratory parameters demonstrated marked metabolic acidosis, sinus tachycardia, and mild ST segment depression. He was admitted with a diagnosis of ischemic heart disease, lactic acidosis and worsening renal failure. His two-day hospitalization was further complicated by large volume diarrhea (? ischemic bowel causing lactic acidosis). He was confused and preemptively intubated and placed on mechanical ventilation. He continued to progress with multiorgan failure and shortly thereafter arrested. The decedent was embalmed, buried, and exhumed 4 months later.

Paraquat and its internal standard, diquat, were extracted from the specimens utilizing a liquid-liquid extraction procedure. Prior to extraction, tissues were homogenized and diluted 1:4 in deionized water. All specimens were deproteinized with TCA, followed by alkalization with NaOH. The aqueous solution was chemically-reduced with an excess of sodium borohydride and extracted with cold diethyl ether. After evaporation of the ether, the extracts were reconstituted in methanol and analyzed with an Agilent 6890 Series GC system equipped with a 5973 MSD. The GC was fitted with a 30 m HP-5MS capillary column with ultra-high-purity helium as the carrier gas at a constant flow rate of 0.4 mL/min. Automated injections were made in split mode with a split ratio of 5:1. The mass spectra were obtained in SIM mode by monitoring *m/z* 96, 148, and 192 for reduced paraquat and *m/z* 108 and 190 for reduced diquat.

Validation studies demonstrated intra-assay and inter-assay %CV values that were less than 12%, intra-assay and inter-assay % accuracy values within 15%, and linearity between 0.25 to 5.0 mg/L. Paraquat was detected at the following concentrations: liver, 1.2 mg/Kg; kidney, <0.5 mg/Kg; spleen, <0.5 mg/Kg; lung, trace; brain, trace; and casket fluid, positive.

In conclusion, paraquat was readily detected in the exhumed remains of a decedent previously exposed to paraquat. The medical examiner determined the cause of death as acute myocardial infarct due to coronary artery atherosclerosis. A contributory cause of death was alcoholic cirrhosis.

Key Words: Paraquat, GC-MS analysis, Exhumed body

P27

Detection of Methadone and Two Major Metabolites in the Hair of Pregnant Women and Their Infants

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Methadone (MD) is considered to be the standard of care in the pregnant, opioid-addicted patient and is metabolized to two primary metabolites, ethylidine-l,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-diphenylpyrroline (EMDP). Detection of MD and its metabolites in the hair of pregnant women and their infants offers the opportunity for studying the relationship between controlled drug administration and disposition of drug and metabolites into hair. The major goal of this study was to examine the relationship between maternal and infant hair concentrations of MD, EDDP, and EMDP after maternal methadone maintenance treatment. Hair samples were collected approximately once per month throughout pregnancy from ten opioid addicted mothers who were enrolled in a methadone treatment program (Methadone maintenance doses = 20-100 mg/day). Hair samples from the infants (n = 4) born to these mothers were collected at the time of delivery. Maternal hair samples (3-cm, segmented) and infant hair samples (non-segmented) were prepared and analyzed for the presence of MD, EDDP, and EMDP by LC/MS/MS. When sufficient quantity existed, both washed and unwashed hair samples were analyzed.

Results revealed considerable variability in the concentrations of MD and metabolites in maternal hair segments (MD, 0.03-214.19 ng/mg; EDDP, 0.10-10.76 ng/mg; EMDP, 0.10-0.20 ng/mg). No statistical difference in analyte concentrations was observed between washed and unwashed hair samples. MD and EDDP were detected in all maternal hair segments corresponding to the period of MD treatment, while EMDP was only occasionally found. MD and EDDP concentrations were typically greatest in those segments of maternal hair found closest to the scalp. Hair concentrations increased throughout pregnancy. Also, MD and EDDP, but not EMDP, were detected in all infant hair samples (MD, 4.81-26.16 ng/mg; EDDP, 5.18-21.47 ng/mg). An important finding was that EDDP concentrations were significantly higher in infant hair than EDDP in maternal hair. Placental metabolism of methadone to EDDP could account for the increased EDDP concentrations observed in infant hair samples. Despite the small number of maternal-infant pairs, it appeared that mothers with higher hair concentrations of MD and EDDP were associated with infants with the highest hair concentrations. This work was supported by NIDA Grants DA R01 09096 and DA R01 12220.

Key Words: Methadone, Hair, Pregnancy

P28

Vitreous Fluid Quantitation of Opiates and Cocaine; Comparison of Calibration Curves in both Blood and Vitreous Matrix

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The specimen of choice for the Montgomery County Coroner's Office (MCCO) as a second matrix for confirmation of positive opiate immunoassay screens is vitreous fluid. Vitreous fluid samples are preferred in this laboratory due to an apparent delay in peak concentration and distribution of rapidly metabolized drugs, 6 monoacetylmorphine (6-MAM), and also for its cleanliness as compared to other matrices such as liver or urine. Due to a large caseload and large batch runs, it is more practical to run only one set of matrix calibrators per batch, for this reason only blood calibrators are used routinely. The question arose as to whether there was a significant matrix effect if quantitation of vitreous fluid was performed using calibrators prepared in a blood matrix. Therefore, a comparison was made between quantitation of vitreous fluid using calibrators prepared in blood matrix and calibrators prepared in vitreous matrix.

Immunoassay opiate kits, purchased from Immunalysis Corporation, were used to screen both blood and vitreous specimens. The results of the screening tests of vitreous and corresponding blood specimens showed consistent results for both blood and vitreous positive and negative controls. Using a solid-phase extraction method, vitreous specimens were confirmed and quantitated using both vitreous and blood calibration curves to compare the quantitative values for any positive drug findings. Specimens were analyzed after BSTFA derivitization by gas chromatography/mass spectrometry (GC/MS) in the positive electron impact mode using selected ion monitoring. Trends in quantitative results for vitreous fluid between blood and vitreous matrix calibration curves and trends between analytes using the two different matrix calibrators are described for 6-MAM, codeine, morphine, hydrocodone, and oxycodone. In the opiate analysis, the majority of concentrations (ug/mL) were higher using blood calibrators to quantitate vitreous specimens. However, most results were within ±20% of each other. The trend followed in all analytes, except morphine. In the case of morphine, blood or vitreous calibration curves did not give consistent results either higher or lower for vitreous concentrations. However, quantitative results that were different between matrix calibrators, and were greater than 20% of each other still could be rounded to the same value. An example of one of the cases analyzed, in which the cause of death was ruled acute heroin and cocaine intoxication, showed the following drug concentrations in vitreous fluid; 6-monoacetylmorphine 0.09 vs. 0.05 jJ-g/mL, morphine 0.07 vs.0.09 ug/mL, and codeine was 0.02 |ag/mL for both blood and vitreous calibration curves, respectively.

In addition to opiates, MCCO is also evaluating the use of vitreous fluid for cocaine and cocaine metabolite analysis as a second matrix for confirmation of positive cocaine metabolite immunoassay screens. Immunoassay screens using cocaine metabolite kits, purchased from Immunalysis Corporation, show consistent results between blood and vitreous fluid positive and negative controls. Currently, the vitreous specimens are being confirmed and quantitated using calibration curves in both blood and vitreous matrices. In addition to the opiate data, the cocaine data will also be presented.

Key Words: Vitreous fluid, Opiates, Matrix effects

P29

The Analysis of Opiates in Hair in Postmortem Toxicology

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Hair is not a specimen routinely collected at autopsy in many medical examiner or coroner's offices. The Montgomery County Coroner's Office routinely collects a hair specimen from every autopsy case. Hair has proven itself to be a viable specimen option for opiate analysis in cases where other specimens were either unavailable or unsuitable for analysis. The practicality of using hair as an alternative matrix is dependent upon the quality of the specimen preparation method. Analysis of hair specimens was successfully completed in this lab by using a combination of two methods and in-house development. The hair specimens were prepared for analysis by using the method by Welch et al. (1993) and extracted by solid phase extraction (SPE) using a method developed by United Chemical Technologies Inc. The number of washes in the specimen preparation was based on the quality of the hair specimen. Following digestion and extraction, the specimens were derivitized with BSTFA +1% TMS and one micoliter was injected onto a gas chromatograph-mass spectrometer in the positive electron impact mode using selected ion monitoring.

Case A concerned the remains of a white female found in a 55 gallon drum buried beneath the edge of a concrete driveway. The cause of death was determined to be asphyxiation. The toxicology section received the following specimens: brain, liver, muscle, spleen, and hair. A spleen homogenate screened positive for opiates. Oxycodone was quantitated in the liver at a concentration of 0.43 µg/g; however, the muscle, brain and spleen specimens proved to be unsuitable for opiate analysis. A hair specimen was analyzed as a second matrix. The hair specimen was washed and sonicated six times due to its poor condition. The decedent's hair had fallen out and collected at the bottom of the drum, and was immersed in a layer of motor oil. The specimen was then digested and extracted by SPE, and the presence of oxycodone was confirmed.

Case B concerned a white female reported to have been found unresponsive at her residence and transferred to the hospital. At the hospital, she tested positive for opiates. Her condition declined and she was pronounced dead four days after admission. According to police reports, the decedent was a known heroin abuser. During the course of her hospital stay she was administered morphine. As a result, the opiate analysis on postmortem specimens was positive for morphine. Because no hospital admission specimens were available, the decedent's hair was analyzed to determine the presence of opiates, specifically 6-monoacetylmorphine (6-MAM), a heroin metabolite. The analysis was positive for 6-MAM, indicating prior heroin exposure, as well as codeine, morphine, and oxycodone. (AAFS 2004)

Case C concerned a white male found floating face down in a local river. It was determined the decedent had been in the water approximately three to four weeks. The decedent was identified by fingerprints and investigators discovered he had been very despondent and had threatened to jump into the river several times. The toxicology section received the following specimens: cavity fluid, bile, gastric contents, brain, liver, spleen, and hair. The cavity fluid screened positive for opiates and free morphine was confirmed in both the cavity fluid and the liver at concentrations of 0.42 µg/mL and 0.74 µg/g, respectively. A hair specimen was analyzed for the presence of 6-MAM, to determine prior heroin use.

Analytical data will be presented from these three cases to illustrate the ability to detect an opiate in hair when other specimens were unsuitable. Quantitation was not performed due to the lack of an appropriate quantitative positive control for hair. Segmental hair analysis was not performed either because time frame of drug use was not of importance or the root end of the hair could not be determined.

Key Words: hair, opiates, postmortem toxicology

P30

Diethyl Ether Distribution in Two Postmortem Cases

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Diethyl ether (ether) is a volatile liquid that was used in the 1800s as an anesthetic agent due to its east of administration and anesthetic effects. Since ether has a high solubility in body fluids and tissue, it produces a slow induction of and a longer recovery time from anesthesia. This, along with its odor and high flammability, especially in combination with oxygen, caused its cessation of use as an anesthetic. Two cases were presented with the presence of ether. The first case was an 18-year-old male found hanging from a basement ceiling brace in a semi-sitting position with a gas mask covering his face. A container of Prestone® starting fluid and a bong were found on the floor close to the body. The second case was a 20-year-old male found unresponsive in his dormitory room. Two black plastic trash bags were secured over his head with a rubber band. Two saturated rags and a clear zip-lock bag containing a white liquid were contained within these trash bags. An almost empty can of Tradco® starting fluid was also found at the scene. Ether concentrations (See Table I) were determined by headspace gas chromatography-mass spectrometry. Selective ion monitoring was performed for *m/z* 31,45 and 59 for ether and 43 and 72 for the internal standard (methyl ethyl ketone). In case #1, the medical examiner ruled that the cause of death was asphyxia due to hanging; the manner of death was undetermined. In case #2, the medical examiner ruled that the cause of death was asphyxia and the manner of death was suicide.

Table I. Distribution of diethyl ether in the presented case.

Specimen	Diethyl Ether	
	Case #1	Case #2
Heart Blood (mg/L)	26	319
Peripheral Blood (mg/L)	24	304
Liver (mg/kg)	36	442
Kidney (mg/kg)	38	490
Bile (mg/L)	29	204
Urine (mg/L)	Not available	123

Key Words: Diethyl Ether, headspace GC/MS, postmortem

P31

A Qualitative Method for the Detection of Helium in Postmortem Blood and Tissues

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We report a qualitative screening method for helium and three recent cases of suicidal asphyxiation by the introduction of helium into a plastic bag placed over the head. A literature search revealed several reported cases of helium asphyxiation but neither blood nor tissue samples were analyzed. In these reports, the case histories were used to determine cause of death. The method described is a quick screen that can be used to detect helium. A Hewlett Packard Model #5182-9646 electronic leak detector operating in gas group #1 calibrated for the detection of Helium (He) and Hydrogen (H_2), was utilized. The detector employs a dual-cell-micro- volume, thermal conductivity system that has a maximum sensitivity of 1 x 10^{-5} mL/sec. The signal magnitude is displayed on-screen. A positive control was prepared by adding 2 mL of blank blood to a 20-mL headspace vial. Helium was then bubbled into the blood for approximately 30 seconds by use of a capillary column. A negative control was prepared by in the same manner except the headspace vial was not introduced to the capillary column. Several different samples were collected for analysis in three medical examiner cases where helium asphyxiation was suspected. These include aorta blood, subclavian blood and lung tissue. Negative case controls were also selected and the same types of specimens analyzed for helium. For cases where the scene findings suggested helium asphyxiation the test was obviously positive. The case controls were obviously negative. Helium was reported as qualitatively present on those specimens that tested positive, hi addition, a result note was included on each report that stated that the laboratory could not rule out the possibility of a false positive due to the presence of hydrogen.

Key Words: Helium, Postmortem, Huffing

P32

Saliva/Plasma Ratios of Cocaine, Ecgonine Methyl Ester and Benzoylecgonine Following Controlled Cocaine Administration

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The objective of this research was to characterize the saliva to plasma (S/P) ratios of cocaine (COC), ecgonine methyl ester (EME) and benzoylecgonine (BE) after controlled subcutaneous COC administration. Comparisons of Cmax, Tmax, and onset and detection times of the three analytes in plasma (P) and oral fluid (OF) also were performed. Nineteen healthy volunteers resided on a secure research unit as part of an IRB-approved study. Participants received low (75 mg/70 kg) and high (150 mg/70 kg) doses of COC separated by three weeks. P and OF (expectoration stimulated by sour candy) were collected for 48 h after drug administration. Following SPE and derivatization with BSTFA (with 1% TMCS), COC, EME and BE were analyzed by GC/MS. Limits of quantification (LOQ) were 2.5 ng/mL for all analytes.

All participants' P was positive for COC by 5 min after both doses. OF was positive for COC by 5 min in 65% (low) and 100% (high) of specimens. Mean onset times of EME and BE in P and OF were similar, approximately 0.1 h (P) and 0.3 h (OF).

Mean COC Cmax in P were 300.3 ± 98.5 ng/mL (low, n= 16) and 637.9 ± 213.8 ng/mL (high, n= 12), while the mean OF COC Cmax were 1322.3 ± 848 ng/mL (low, n=14) and 3130.7 ± 2228.2 ng/mL (high, n=8). Mean EME Cmax in P after the low dose was 47.4 ± 11.5 ng/mL (n=15), compared to 124.4 ± 68.1 ng/mL (n=14) after the high dose. Mean OF EME Cmax was 154.5 ± 84.8 ng/mL (n=16) after the low dose, increasing to 395.3 ±271.6 ng/mL (n=12) after the high dose. MeanBE Cmax inP were 321.3 ±71.1 ng/mL (low, n=15) and 573.8 + 166.5 ng/mL (high, n=l0), and mean BE Cmax in OF were 154.7 ± 100.3 ng/mL (low, n=l7) and 308.0 ± 163.8 ng/mL (high, n=12). After both doses in matched subjects, COC and EME Cmax were significantly higher in OF than P, while the opposite was true for BE Cmax. Mean COC Tmax for P and OF in matched subjects were 0.56 ± 0.21 h and 0.94 ± 0.52 h (low, n=14) and 0.62 ± 0.33 h and 0.95 ± 0.42 h (high, n=l 1), respectively.

In both matrices, > 93% of participants were positive for all analytes at 8 h. In general, detection times were COC < EME < BE, with no statistical differences between P and OF in subject-matched specimens.

Specimens were grouped into three timeframes (0.08- 0.25 h, 0.5-8 h, and 11.5-48 h), and S/P ratios were described as follows: less than 1, between 1 and 2, between 2 and 3, and greater than 3. After the low dose, the highest % of specimens had COC S/P ratios between 1 and 2 (40%) in the 1st timeframe, > 3 (48%) in the 2nd timeframe, and < 1 (42%) in the 3rd timeframe. COC S/P ratios following the high dose were similar in the first two timeframes, while in the 3rd, 44% of S/P ratios remained > 3. After the low dose, 90% of EME S/P ratios were < 1 during the 1st timeframe, while 52% and 66% were between 1 and 2 during the 2nd and 3rd timeframes, respectively. A similar trend was observed for EME S/P ratios after the high dose. Greater than 84% of BE S/P ratios were < 1 throughout all timeframes following both doses.

These COC and metabolite data collected after controlled COC administration increase our understanding of the disposition of COC, EME and BE in OF and P.

Key Words: S/P ratios; cocaine; oral fluid

P33

Preliminary Observations of the NLCP Hair Pilot Performance Testing Program: Confirmatory Analysis of Stimulants

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Introduction: In 2000, SAMHSA's National Laboratory Certification Program (NLCP) began a Pilot Performance Testing (PPT) Program to develop appropriate hair samples and assess the participant laboratory confirmatory testing capabilities for this matrix. Since its inception, this PPT program has provided 36 samples containing amphetamine/methamphetamine and 46 samples containing cocaine analytes to the 5 to 13 participating laboratories.

Objective: To retrospectively evaluate the effectiveness of the NLCP PPT program: 1) to develop hair performance testing samples, and (2) to assess the ability of laboratories to accurately detect and quantify drugs of abuse in hair.

Methods: Hair included for analysis was either collected from known drug users or fortified with drugs of abuse by NLCP protocols. Samples were sent to the laboratories in a blinded fashion in 8 cycles during a five-year period. Approximately 100 mg of each hair sample was stored and shipped overnight at ambient temperature prior to analysis. As directed by the NLCP, some hair samples were subjected to each laboratory's decontamination (wash) procedures prior to extraction and all were analyzed by their standard operating procedures for confirmatory testing. Some specimens were sent to the laboratories in multiple cycles. Generally, fortified samples were targeted for analyte concentrations between 0.5 to 2 times the SAMHSA proposed confirmatory cutoffs for hair specimens as published in the Proposed Revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs (69 Fed. Reg. 19673, April 13, 2004).

Results: The following table summarizes: 1) the regression analysis (first 5 rows) comparing the achieved results to the targeted values [95% confidence interval (CI) and unweighted y-intercept option] using all data points; 2) the statistical analysis calculations (95% CI) for the proposed cutoff concentrations (last 5 rows).

LINEAR REGRESSION ANALYSIS FOR TARGET CONCENTRATIONS (pg/mg)					
Range	40-600	150-5000	350-10000	50-1500	50-200
Slope	1.30	0.46	0.84	1.35	2.98
y-intercept	-92	286	636	0	2
r^2	0.591	0.693	0.498	0.294	0.573
N	87	127	271	184	31
STATISTICAL ANALYSIS AT CUTOFF CONCENTRATION (pg/mg)					
Range	30-247	110-599	200-1398	13-150	101-483
Mean (cutoff)	192 (300)	285.8 (300)	943.4 (500)	47.0 (50)	212.9 (50)
Median	239	280	1080	44	206
%CV	41.7	44.3	36.0	66.6	45.1
N	15	30	28	19	13

Conclusions: A summary of observations include: 1) Overall precision and accuracy of hair analysis among laboratories is low given CVs > 20% and large deviations of mean/median values to cutoffs; 2) Limited data are available to support intra-laboratory precision; 3) Regression analyses (e.g. r^2 <0.8, and highly variable y-intercepts and slopes) demonstrate that the overall performance of the NLCP hair PPT program to incorporate stimulants into hair specimens and the analytical laboratory performance does not currently achieve standards maintained by the established urine PT programs; 4) Correlation to target concentrations varies but the demonstrated precision confines the identification of a performance-based cutoff with precision limits that would emulate urine testing programs; 5) While results from the NLCP hair PPT program are encouraging and have improved over time, the program will require continued effort to improve both laboratory analytical performance and the NLCP ability to prepare appropriate hair PT samples.

Key Words: Hair, Performance Testing, Workplace Drug Testing

P34

Preliminary Observations of the NLCP Oral Fluid Pilot Performance Testing Program: Confirmatory Analysis of Stimulants

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Introduction: In 2000, SAMHSA's National Laboratory Certification Program (NLCP) began a Pilot Performance Testing (PPT) Program to develop appropriate oral fluid samples and assess the participant laboratory confirmatory testing capabilities for this matrix. Since its inception, the NLCP PPT program provided 17 PT samples for analysis of amphetamine, 23 for methamphetamine, 31 for benzoylecgonine, 13 for cocaine, and 9 samples for MDMA, MDA, and MDEA. The number of laboratories participating in this program varied from 6 to 17 laboratories.

Objective: To retrospectively evaluate the effectiveness of the NLCP PPT program: 1) to develop oral fluid performance testing samples, and 2) to assess the ability of laboratories to accurately detect and quantify drugs of abuse in oral fluid.

Methods: Normal human oral fluid was collected from a drug free donor and spiked at concentrations that ranged from 0.25 to 32 times the SAMHSA proposed confirmatory cutoff, as published in the Proposed Revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs (69 Fed. Reg. 19673, April 13, 2004). Once prepared, the samples were stored frozen in silanized 4 mL capped vials and shipped frozen by overnight delivery. Some samples were sent to the laboratories on multiple occasions. All testing was analyte directed by confirmatory test methods.

Results: The following table summarizes: 1) the regression analysis (first 5 rows) comparing the achieved results to the targeted values [95% confidence interval (CI) and unweighted y-intercept option] using all data points; 2) the statistical analysis calculations (95% CI) for the proposed cutoff concentrations (last 5 rows).

Value	Amphet	Methamp	MDMA	MDA	MDEA	Cocaine	BZE
LINEAR REGRESSION ANALYSIS FOR TARGET CONCENTRATIONS (ng/mL)							
Range	25-160	25-1600	25-100	25-100	25-100	4-40	0-200
Slope	1.20	1.03	0.87	0.87	0.91	0.78	0.94
y-intercept	-10.11	-2.02	1.45	-0.09	1.97	1.53	0.40
r^2	0.827	0.941	0.839	0.859	0.866	0.678	0.903
N	170	220	81	78	33	100	241
STATISTICAL ANALYSIS AT CUTOFF CONCENTRATION (ng/mL)							
Range	33.7-76.6	34.7-65	13-54	10.5-55.9	36-52.6	2.1-19.3	1.9-16
Mean (cutoff)	49.2 (50)	49.9 (50)	44.4 (50)	42.5 (50)	46.6 (50)	8.1(8)	6.9 (8)
Median	49.8	49.5	45.5	42	49	7.8	6.9
%CV	15.7	13.8	17.9	21.0	12.3	40.2	26.9
N	60	60	27	26	11	25	69

Conclusions: A summary of observations include: 1) Regression analyses (e.g., r^2 >0.8, y-intercepts less than ± 10 ng/mL from the origin, slopes between 0.8 and 1.2) demonstrate the overall performance of the NLCP PPT program to prepare samples in oral fluid and the participating laboratories to accurately achieve expected values for all stimulant analytes, except cocaine. Cocaine's lower regression analyses may be attributed to degradation during storage or during laboratory analytical preparations; 2) The mean and median values for samples containing analytes at or near the proposed SAMHSA confirmatory test cutoff were within 15% of that cutoff; however, only four of the seven analytes had a %CV of <20%; 3). While results from the oral fluid PPT program for stimulants are encouraging and have improved over time, the program will require continued effort to improve both laboratory analytical performance and the NLCP capacity to prepare appropriate oral fluid PT samples.

Key Words: Oral Fluid, Performance Testing, Workplace Drug Testing

P35

Methamphetamine and Amphetamine Disposition in Human Sweat Following Controlled Oral Methamphetamine Administration

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Methamphetamine (MAMP) is widely abused for its euphoric and performance enhancing effects. The study objective was to determine the disposition of MAMP and amphetamine (AMPH) in human sweat following controlled oral administration. Healthy volunteers provided informed consent to participate in this NIDA Institutional Review Board approved protocol and resided on a secure research unit for 10 weeks. Participants (n=8) received 4 daily 10 mg (low) oral doses of sustained release (d)-MAMP-HCl within 7 days. After a 4-week interval, 5 of 8 participants also received 4 daily 20 mg (high) oral doses. PharmChek™ sweat patches (n=682) were applied weekly during a 3-week washout period, and during and after drug administration. MAMP and AMPH were isolated by SPE followed by GC/MS with a LOD and LOQof2.5ng/patch.

Weekly sweat patches collected prior to the first MAMP administration reflected the excretion of previously self-administered amphetamines. No patch (n=38) was positive for MAMP or AMPH at the LOQ. Short-term sweat patches worn for 2, 4, or 15 h during the first 24 h after low and high doses were evaluated to determine the time of first drug detection. MAMP was detected as early as 2 h following low and high doses. However, AMPH was only present during high dose administrations. Of 48 short-term patches, half contained MAMP or AMPH above the method's LOQ. AMP was detected in only 5 patches and never without concurrent MAMP. In patches worn from 8 to 23 h following each of 4 consecutive daily MAMP administrations, concentrations did not indicate accumulation following multiple low or high doses. 93% and 62% of weekly sweat patches applied for 7 days during low and high MAMP administration were positive for MAMP. In comparison, 100% and 75% of these weekly patches were above the LOQ for AMPH. Mean MAMP concentrations for weekly patches during dosing were 71.0 ± 48.7 ng/patch (range 0 - 175.3, n=14) for the low dose and 217.1 ± 213.7 ng/patch (range 0 - 606.5, n=8) following the high dose. Mean AMPH concentrations for the same weekly patches were 17.4 ± 10.2 (range 6.5 - 40.5, n=14) and 43.5 ± 32.8 ng/patch (range 0 - 83.4, n=8) following low and high doses.

SAMHSA requirements for a confirmed positive sweat test include MAMP of >25 ng/patch and AMPH at a concentration greater than or equal to the method's LOD. 85.7% of all weekly patches (n=14) worn during low dose administration were positive under SAMHSA guidelines as compared to 62.5% of all weekly high dose patches (n= 8).

Mean MAMP concentrations for weekly patches applied 3 days after the last low and high MAMP administrations were 5.2 ± 6.4 ng/patch and 9.7 ± 7.7 ng/patch, respectively. None of these weekly patches applied one week after dosing fulfilled the SAMHSA requirements for a positive MAMP sweat test.

These data contribute to our understanding of MAMP and AMP excretion in sweat and provide controlled administration data for the interpretation of sweat testing results.

Key Words: Methamphetamine, Sweat

P36

Evaluation of Software Designed to Automate Quality Control for GC/MS Drugs of Abuse Confirmations

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Routine confirmations of drug use using gas chromatography/mass spectrometry (GC/MS) require analytical boundaries for quality control to ensure batch acceptability. These boundaries range from chromatographic peak shape considerations to analytical accuracy. Standard operating procedures for toxicology laboratories typically specify parameters for judging these quality control criteria. However, most GC/MS software programs require users to manually interpret data and make the appropriate adjustments to the batch, or the programs may perform minimal interpretive tests and leave other evaluations to the user. A software program designed to not only perform these tests for quality control acceptability but also take actions upon "Pass" or "Fail" determinations was evaluated for performance in a laboratory setting. The ability of the software to assess user-determined criteria and then take the desired steps to correct any deficiencies was compared to analytical software that did not have these capabilities. Overall, this program appears to offer features that may be of value for laboratories that use GC/MS for substance abuse analysis.

P37

The Incorporation of Delta-9-tetrahydrocannabinol and Its Metabolite, ll-nor-9-carboxy-delta-9-tetrahydrocannabinol, into Hair

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Hair pigmentation has been shown to play a role in the concentration of drug incorporated into hair. The binding of basic drugs such as cocaine, codeine and amphetamine to melanin is believed to produce a hair color effect through both ionic and non-ionic interactions. The purpose of this study was to evaluate the effect of hair pigmentation on the incorporation of the non-basic drugs, Δ^9-tetrahydrocannabinol (THC) and 1 l-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THCA), into hair. Long-Evans (LE) rats were administered THC at 30 mg/kg (oral gavage; n=5) daily for 28 days. Animal hair was shaved just prior to the first administration, and newly grown hair was collected fourteen days after the final dose. Pigmented and non-pigmented hair was collected and analyzed by gas chromatography/mass spectrometry operated in negative chemical ionization mode. Concentrations of THC and its metabolite, THC A, in the newly grown rat hair were compared with area under the concentration versus time curves (AUCs) of the drugs in the rat plasma to determine incorporation rates (ICR). The hair concentrations for THC were 191.4 ± 48.6 pg/mg in pigmented hair and 239.6 ± 68.4 pg/mg in non-pigmented hair. THCA hair concentrations were 22.2 ±3.5 pg/mg in pigmented hair and 19.4 ± 5.2 pg/mg in non-pigmented hair. Differences between the means of THC or THCA in pigmented and non-pigmented rat hair were not significant ($p > 0.05$). Following 30 mg/kg THC by oral gavage, plasma AUCs were 154.9 [µg-min/ml and 138.8 (µg-min/ml for THC and THCA, respectively. After normalizing for the plasma concentrations, the incorporation of THC into Long-Evans rat hair was one order of magnitude greater than THCA. The data presented here confirms that THC is more readily incorporated into hair than THCA, and that melanin does not play a role in the incorporation of THC and THCA into pigmented hair versus non-pigmented hair.

Key Words: delta-9-tetrahydrocannabinol, hair, incorporation

P38

Quantitative Analysis of Gamma-Hydroxy-Butyrate (GHB) in Urine by Direct Analysis in Real Time (DART™) Time-of-Flight Mass Spectrometry (TOF-MS)

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DART™ stands for "Direct Analysis in Real Time". A new ion source, DART™ allows for the direct detection of chemicals on surfaces, in liquids, and in gases without the need for sample preparation. The ion source is open to the atmosphere, and does not require a vacuum, the use of high voltages or solvent sprays. Samples are simply placed into the ionization stream. The ionization mechanism is based upon the reactions of excited-state species with reagent molecules and polar or non-polar analytes. When coupled with a TOF-MS, accurate mass assignments and therefore analyte identifications are realized.

Gamma-hydroxybutyrate (GHB) is used as a recreational drug, to facilitate sexual assaults, and as a body-building supplement. Prescriptions for the treatment of cataplexy in patients with narcolepsy are also available.

Screening for GHB in urine specimens usually requires the use of a liquid-liquid or solid phase separation technique before GC or GC-MS analysis. A conversion to the lactone analogue (GBL) or inclusion of a derivatization step is often necessary for gas chromatographic analysis. Generally, a cut-off of 10 ug/mL in urine is employed to distinguish between endogenous and exogenous GHB.

The primary purpose of this exploratory study is to determine whether DART™ coupled with TOF-MS is a viable screening technique for the near-instantaneous detection of exogenous GHB in urine and other matrices.

GHB-free synthetic urine aliquots were spiked with GHB to achieve a concentration range of zero to 800ug/mL. Similar standards were also prepared in methanol and normal human urine.

Sampling was achieved by simply dipping a glass capillary tube into the specimen, and then "waving" the tube in the ionization stream of the AccuTOF™ with DART™ for several seconds. Variability in sample presentation to the ionization source was negated by the use of a deuterated internal standard (d6-GHB). The GHB anion ($C_4H_7O_3$") was monitored at *m/z* 103.039, along with the corresponding ion for the deuterated analogue. A ratio of analyte/internal standard was calculated.

Analysis of GHB-spiked methanol samples yielded a linear response curve from zero to 800 ug/mL (r^2=l .00). The LOD for GHB in methanol was 1.0 ug/mL or better. Analysis of GHB-spiked urine samples yielded a linear response curve from zero to 200ug/mL (r^2=0.995). The LOD for GHB in urine was 5 ug/mL or better.

This investigative study demonstrates that DART™ coupled with TOF-MS is a viable method by which to screen for GHB in urine and other matrices. Further work is necessary to fully characterize the interconversion of GHB to its lactone analogue GBL, matrix effects, and possible interferences.

It is anticipated that improvements in the DART™ ionization efficiency and sample introduction will lower detection limits from the demonstrated parts per million range, making the instantaneous screening of biological specimens for GHB and other drugs a very real possibility.

Key Words: GHB, DART, TOF-MS

P39

Detection Rates, Dose-concentration Relationships, and Reproducibility of Cocaine, Benzoylecgonine, and Ecgonine Methyl Ester in Human Sweat Following Controlled Cocaine Administration

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Alternative biological matrices such as sweat provide a less invasive method of monitoring illicit drug use. Sweat also offers the advantage of a longer detection window than urine, oral fluid or plasma. Drugs excreted in sweat can be collected with the PharmCheck® sweat patch. The Substance Abuse and Mental Health Services Administration (SAMHSA) has proposed a screening cutoff concentration of 25 ng/patch for cocaine metabolites in patches worn for three to seven days. The proposed confirmation cutoff is 25 ng/patch of cocaine or benzoylecgonine (BE).

A controlled drug administration study was conducted to comprehensively evaluate the excretion of cocaine in sweat. Fifteen volunteers with a history of cocaine use provided informed consent to participate in this IRB-approved study. Nine participants completed the entire study, receiving three low doses (75 mg/70kg) of subcutaneous cocaine HC1 within one week and, three weeks later, three high doses (150 mg/70kg) following the same schedule. PharmChek® sweat patches (n=1390) were collected before, during and after controlled dosing and analyzed by gas chromatography-mass spectrometry.

Cocaine was the primary analyte detected in sweat. One quarter of the patches were positive at the method's limit of quantification (LOQ) of 2.5 ng/patch; 7% were positive at the proposed SAMHSA cutoff. Almost 60% of 342 positive patches contained only cocaine; less than 20% contained cocaine, BE and ecgonine methyl ester (EME). BE and EME were detected above the LOQ in 21% and 37% of positive patches, respectively. In patches containing both BE and EME, the mean BE concentration was 14.2 ± 23.4 ng/patch and the mean EME concentration was 13.4 ± 13.3 ng/patch. At the method LOQ, EME concentrations exceeded those of BE in 53% of patches containing both analytes. In these patches, the average BE/EME ratio was 1.1 (median - 0.9) due to elevated BE concentrations in some patches. One-way analysis of variance indicated no statistically significant difference in mean ratios in patches worn for less than 24 hours (mean = 1.0 ± 0.7), 1-6 days (mean = 1.3 ± 0.6), and weekly (mean = 1.2 ± 0.7; D=0.05). There were no statistically significant dose-concentration relationships for any analyte in patches worn for 15 hours during the day of dosing or patches worn for the entire dosing week. Duplicate patches, used to evaluate reproducibility, had matching qualitative and quantitative cocaine, BE, and EME results in 79.4%, 87.7% and 85.3% of cases, respectively. This controlled cocaine excretion protocol indicated that sweat testing is an effective and reliable method of monitoring cocaine exposure.

Key Words: Cocaine, Excretion, Sweat

P40

Nicotine, Cotinine, Trans-3'-Hydroxycotinine, and Norcotinine as Biomarkers of Tobacco Smoke Exposure in Oral Fluid of Pregnant Smokers

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Nicotine, the major addictive component in tobacco, is rapidly metabolized in humans and is a potential risk factor during pregnancy. Nicotine is extensively metabolized by cytochrome P450 (CYP2A6) to cotinine. Cotinine is further metabolized to trans-3'-hydroxycotinine (hydroxycotinine) and other minor metabolites. The objectives of this study were to determine nicotine, cotinine, hydroxycotinine, and norcotinine concentrations in oral fluid of pregnant smokers and to evaluate nicotine and metabolites as biomarkers of cigarette smoking.

This clinical study was approved by the JHBMC and the NIDA Institutional Review Boards. Participants were opioid dependent and tobacco smoking pregnant women of less than 28 weeks gestation enrolled in a methadone maintenance treatment program. All participants provided written informed consent and were paid for their participation. Sixteen participants were divided into two groups based on self-reported smoking habits; eight light smokers (LS) who smoked <10 cigarettes/day and eight heavy smokers (HS) who smoked >20 cigarettes/day. Oral fluid specimens (n=415) were collected three times per week throughout the pregnancy with ad libitum cigarette smoking. Specimens were analyzed by SPE followed by GC/MS/EI in SIM mode.

Nicotine, cotinine, and hydroxycotinine were found in all oral fluid specimens. The median concentrations of nicotine, cotinine, and hydroxycotinine in oral fluid for the LS ranged from 241.1 - 622.0 (overall mean ± SD, 575.4 ± 677.8 ng/mL), 80.6 - 387.5 (overall mean 219.7 ± 110.9 ng/mL), and 14.4 - 117.7 (overall mean 62.7 ± 42.6 ng/mL), respectively. The median concentrations of nicotine, cotinine, and hydroxycotinine in oral fluid for the HS ranged from 146.5 -1372.2 (overall mean 798.3 ± 821.6 ng/mL), 66.0 - 245.8 (overall mean 168.0 ± 74.6 ng/mL), and 38.3 -184.4 (overall mean 111.0 ± 58.7 ng/mL), respectively. Norcotinine was detected in 38 of 415 oral fluid specimens, from seven of 16 participants (3 LS, 4 HS), with a range from 5.2 to 12.7 ng/mL. Salivary cotinine and hydroxycotinine concentrations were significantly correlated in LS ($r=0.55$, $p<0.01$) and HS ($r=0.74$, $p<0.01$). However, neither analyte concentration correlated to nicotine concentrations in LS. Although salivary nicotine concentrations were significantly correlated to cotinine ($r=0.26$, $p<0.01$) and hydroxycotinine concentrations ($r=0.36$, $p<0.01$) in HS, individual variability precluded prediction of nicotine oral fluid concentrations from metabolite results. Mean nicotine, cotinine, and hydroxycotinine concentrations from 11 participants (4 LS, 7 HS) were not significantly different between the 2nd and 3rd trimesters.

In conclusion, our study describes assessment of nicotine metabolic status in pregnant smokers with a non-invasive oral fluid collection method and quantification of nicotine, cotinine, hydroxycotinine, and norcotinine concentrations. Cotinine and hydroxycotinine in oral fluid were significantly correlated in both LS and HS and are good biomarkers of tobacco exposure. It is not necessary to monitor norcotinine, due to its low prevalence in oral fluid specimens from pregnant smokers.

Key Words: Nicotine, Cotinine, trans-3'-Hydroxycotinine, Norcotinine, Oral fluid

P41

Methylation of 3,4-Methylenedioxymethamphetamine (Ecstasy, MDMA) in Formalin Fixed Human Liver Tissue

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Drugs containing secondary aliphatic amines are methylated in the presence of formaldehyde. We evaluated the stability of MDMA (Ecstasy, 3,4-methylenedioxymethamphetamine) in formaldehyde-fixed liver. Randomly chosen human liver pieces (100-200 mg) were injected with 2 µL (2 µg) of MDMA HC1. The liver pieces in centrifuge tubes were covered with 200 µL of formalin solution (20% v/v), held at room temperature for 24 h, and then homogenized. The resulting suspension was sonicated for 5 min and then centrifuged. Controls consisted of substitution of 200 µL of water in place of formalin solution. Supernatant aliquots (10 µL) were added to 500 µL of 0.1% formic acid in acetonitrile for mass spectrometric analysis. 3,4-Methylenedioxy-N,N-dimethylamphetamine (MDDA) standard was synthesized by dissolving MDMA HC1 in 20% formaldehyde solution at room temperature overnight. Multistage positive ion electrospray mass spectra recorded in MS, MS^2 and MS^3 modes were used to confirm the N-methylation of MDMA by formaldehyde treatment both in solution and when MDMA was injected into liver tissue prior to formaldehyde fixation. In formalin-fixed liver from an MDMA positive case, MDDA was identified by multistage MS analysis. Toxicologists are cautioned that MDMA concentrations in formalin-fixed tissue may be diminished as a result of methylation of MDMA. Identification of both MDMA and MDDA in formalin-fixed samples may provide a practical indicator of the presence of MDMA.

Key Words: MDMA, Formalin, Mass Spectrometry

P42

Feasibility of Extraction and Quantitation of Δ^9-Tetrahydrocannabinol in Body Fluids by Stir Bar Sorptive Extraction (SBSE) and Fast GC/MS

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Proposed revisions to the DHHS Mandatory Guidelines for Federal Workplace Drug Testing Programs (Federal Register, April 13, 2004) include a confirmatory cutoff level of 2.0 ng/mL for THC (parent) in oral fluid. The relatively low cutoff level and small specimen volume (versus the corresponding parameters for urine drug testing) present a challenge to workplace drug testing laboratories. The GC-MS confirmatory procedures for THCA commonly used in certified workplace urine drug testing laboratories lack the sensitivity needed for this application.

We have completed preliminary studies that demonstrate the feasibility of extracting THC from an aqueous matrix using Stir Bar Sorptive Extraction (SBSE) followed by thermal desorption into the injection port of a standard single quadrupole electron impact GC-MS to achieve detection limits less than 1.0 ng/mL. The specimen is diluted with water + methanol and extracted with a stir bar coated with a nonpolar polydimethylsiloxane phase. THC is extracted with high efficiency and minimal matrix interference with no additional sample cleanup needed. THC is introduced onto the GC column by thermal desorption followed by fast GC/MS and selected ion monitoring detection to achieve detection limits in the low ng/mL range.

This technique appears to offer significant advantages of low cost, high extraction efficiency, minimal sample preparation, and compatibility with standard electron impact GC-MS instruments already in common use in workplace drug testing laboratories. With further refinements and optimization for the oral fluid matrix, it may be possible to satisfy the routine confirmatory testing requirements for oral fluid without having to rely on GC-MS-MS or LC-MS-MS techniques to achieve the necessary sensitivity and avoid matrix interference.

Key Words: SBSE, THC, GC-MS

P43

Marijuana and Driving: A Retrospective Study of New Mexico Drivers

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Marijuana (Cannabis) is one of the most commonly abused illicit drugs in the world. The main active chemical is Delta-9 tetrahydrocannabinol (THC) and its primary inactive metabolite is 1 l-nor-9 - carboxy Δ^9-tetrahydrocannabinol (THCA). Membranes of certain nerve cells in the brain contain protein receptors that bind to THC. Once securely in place, THC kicks off a series of cellular reactions that ultimately lead to the euphoric high users experience when they smoke. During the last four years, the State of New Mexico Dept. of Health Scientific Laboratory Division, Toxicology Bureau has confirmed marijuana increasingly amongst DUI cases. 54 cases were reported in 2002, 282 cases in 2003, 329 cases in 2004 and 102 cases for 2005 (Jan. to May). 85% were male and 15% were female in 2002, 84% male and 16% female in 2003, 82% male and 18% female in 2004, and 79% male 21% female in 2005. Average age in 2002 was 31 yrs (range 20-63), in 2003 it was 30 yrs (range 17-63), in 2004 it was 29(range 16-69) and in 2005 it was 27 yrs (range 17-59). A drug of abuse screen was performed on all drivers with alcohol levels less than 0.08 gm/l00mL. The immunoassay screen included six classes of drugs: Benzodiazepine, Cocaine, Methamphetamine, PCP, THC, and Opiates. Quantitative analysis was performed using solid phase extraction and analyzed on negative CI GC/MS with SIM monitoring. Blood concentrations were quantified with a mean of 6 ng/mL THC, 70 ng/mL THCA for cases in 2002, 2003 cases had a mean of 8 ng/mL THC and 41 ng/mL THCA, 2004 cases had a mean was 7 ng/mL THC and 41 ng/mL THCA, and in 2005 the mean was 9 ng/mL THC and 41 ng/mL THCA. There were a wide range of reasons for stop/arrest, which included accident, erratic driving, DRE evaluations (95% accuracy rate in determining based on DRE training) and drug facilitated sexual assault cases. Police report data summarizes that smokers were aware of their impairment, causing them to slow down and become more cautious. Reaction times and attention spans were subsequently affected. Frequently, driving speeds were experientially "faster" than normal. Data from the New Mexico Sentencing Commission was also obtained to correlate with the arrest data to determine the amount of cases being charged. Few cases were actually prosecuted, as most were dismissed.

Key Words: Marijuana, Driving Under the Influence, GC-MS

P44

Comparison of Electron Impact lonization and Positive Ion Chemical lonization Gas Chromatography/Quadrupole Mass Spectrometry for Confirmation and Quantification of Benzoylecgonine in Oral Fluid

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Introduction

Methods were developed to determine benzoylecgonine (BE), a major metabolite of cocaine, in oral fluids using gas chromatography and electron impact (El) and positive ion chemical ionization (PCI) with a single-stage quadrupole mass spectrometer (GC/MS). GC/MS was used due to current widespread implementation of this methodology in many laboratories for routine confirmation of the presence of common drugs of abuse in urine samples. Both the El method and the PCI method were validated according to a proposed method validation procedure. The results of these validations were compared.

Results

BE in oral fluid was analyzed as the hexafluoroisopropyl derivative (HFIP). Derivatization improves the chromatographic properties of the BE, as well as increasing the abundance of higher-mass ions. A deuterated internal standard, BE-D3, was used for quantification, and a single point calibrator established the calibration range. Initial method parameters were developed using El selected ion monitoring (SIM). The El method provided satisfactory linear range, precision, and specificity, and ion ratios of the area of the qualifier ions to the quantitation ion were within + 20% of the target ratio. However, the signal-to-noise (S/N) ratio for the confirmation ion *(rn/z* 439) at a concentration of 1.0 ng/mL was 17:1 RMS; therefore, PCI was explored as a means of improving signal-to-noise. The PCI method was optimized for reagent gas type and chromatographic performance.

Both methods were validated, and this validation included linearity and carryover studies, and an evaluation of precision. Ion ratio confirmation analysis, using the area of the qualifier ion related to the area of the quantification ion, showed that these ratios for BE and BE-D3 were within + 20% of the respective ratios for the calibrator across the linearity range.

Conclusions

Both EI-SIM and PCI-SIM can be used for the confirmation and quantification of BE in oral fluids. EI-SIM offers ease of use and satisfactory linear range. Alternatively, PCI-SIM offers lower noise and sensitivity and it can be optimized to provide qualifier ions for ion ratio confirmation. Use of PCI-SIM, however, requires reagent gas and slower GC analysis times to separate matrix from target components.

Key Words: Oral Fluids, Benzoylecgonine, GC/MS

P45

Extraction of Benzodiazepines Using the Cerex Polychrom Clin II SPE Cartridges in Blood and Urine and Analysis by GC/MS in Selective Ion Monitoring (SIM) Mode

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Benzodiazepines are a group of compounds prescribed for a variety of conditions including insomnia, sedation, epilepsy, and anxiety. There are many formulations of benzodiazepines available in the United States; consequently they are an analytical challenge. The following method can detect a majority, if not all the benzodiazepines and/or metabolites currently available.

Among the Driving Under the Influence of Drugs (DUID) and Sex assault cases submitted to the Phoenix Police Department (PPD) for testing, the broad class of benzodiazepines is found 25 % and 20 % of the time, respectively.

The following poster will detail extraction and analysis of 18 different benzodiazepines in urine and 21 in blood. Extraction efficiencies with the Cerex Polycrom Clinll columns were found to be 70% and above. Derivitization with BSTFA and analysis with Agilent 5890/5972(urine) and 5890/5973 (blood) instruments equipped with DB 5, ISmeter column (urine) and DB 5, 30 meter column (blood) in Selective Ion Monitoring (SIM) mode have proved to be successful. Linearity for common benzodiazepines was determined to be 10-1000 ng/mL. Two years of quantitative data in both blood and urine will be presented. The most common benzodiazepine in blood was nordiazepam and in urine was oxazepam. Patterns and predictions of benzodiazepines(BZD's) in blood and urine will be discussed. Internal standard choices/considerations in both blood/urine. Custom reports templates reduce printouts to six pages per sample (more than two drugs per page). Informative poster for laboratories having difficulty confirming benzodiazepines.

P46

Analysis of Samples for Δ^9Tetrahydrocannabinol: a Possible Analytical Problem

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Cannabis saliva in various forms is widely used in Europe and America. Users of cannabis describe a range of effects, euphoria, relaxation drowsiness and perceptual changes. Δ^9 - tetrahydrocannabinol (THC) is the primary psychoactive constituent of preparations derived from Cannabis sativa. The measurement of THC in blood is useful where there is a suspicion that recent use of cannabis may have contributed to a person's unusual behaviour, though analysis may be difficult due to the low expected concentrations of THC and its short half-life.

In our laboratory during recent work for method development of our own THC assay we found that when solutions containing THC were dried down (a step universally required during preparation of samples for analysis) the THC in solution readily binds to glassware.

In a series of experiments a known amount of THC and a deuterated THC (THC-d3) were dried down in tubes. The THC and THC-d3 was derivatised using N, O Bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA/TMCS) before detection using gas chromatography / mass spectrometry (GC/MS). We were able to demonstrate that 56.9% of THC and 57.0% of THC-d3 were lost when using ordinary glass tubes when compared to tubes, which had been previously silonized. On investigating the literature for reports of this sort of loss we established that there had been some discussion of this problem in the 1980's, but there are no recent papers in which a method for measurement of THC is described within which this problem is discussed. The mass of THC and THC-d3 being lost in non silonized glassware is greater than half of that added when compared to the amounts detected in silonized glassware, a result we believe to be highly significant. We would reiterate, on the basis of these results, that there is a requirement for glassware to be silonized in any protocol where THC extracts are dried down during the course of analysis. This will lead to significant improvement in recovery and thereby will lead to improved limits of detection and quantification.

P47

Optimization of Glucuronide Hydrolysis for Improved Recovery of 11-Hydroxy-Δ^9-Tetrahydrocannabinol in Urine

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A GC/MS method was developed to simultaneously quantify three cannabinoid analytes [Δ^9-tetrahydrocannabinol (THC), ll-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and ll-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH)] in urine. The method employs an enzymatic and base (NaOH) hydrolysis to optimize recovery of cannabinoids. The enzymatic hydrolysis was a modification of the Kemp *et al.* (1995) procedure using |3-glucuronidase from *E. coli.* The method was subsequently applied to urine specimens collected during a clinical study of heavy cannabis users. The NIDA Institutional Review Board approved the clinical study and participants provided informed consent. During method validation it was discovered that diluting specimens ranging in concentration from 5 to 115 ng/mL (N=69) 1:2 in blank urine were consistently returning increased 11-OH-THC concentrations (average +39%) compared to results from undiluted specimens. Further investigation demonstrated improved 11-OH-THC recovery with modified hydrolysis conditions. This study reports the results of experiments developed to enhance the recovery of 11-OH-THC from urine.

The source of enzyme *(E. coli* vs. *H. pomatia),* amount of enzyme (2500, 5000, and 10,000 U/mL of urine), and hydrolysis temperature (37, 45, 55 and 65°C) were evaluated. Additional studies were performed to determine the effect of reversing the enzyme and base hydrolysis steps on specimens with high concentrations of 11-OH-THC and THCCOOH. In one assay, enzyme hydrolysis preceded alkaline hydrolysis and in the other, base hydrolysis was followed by enzyme hydrolysis. A 16-hour enzymatic hydrolysis time was used throughout.

Helix pomatia hydrolyzed specimens yielded no detectable 11-OH-THC at 37°C and showed an 84.5% decrease in recovery relative to *E. coli* hydrolyzed specimens at 45°C. 11-OH-THC concentrations increased an average of 47.2% in undiluted specimens and 41.3% in 1:2 diluted specimens when *E. coli* (3-glucuronidase enzyme amount was increased from 2500 to 5000 U/mL These concentrations also increased an average of 29.3% and 14.8% when enzyme amount was increased from 5000 to 10,000 U/mL. Hydrolysis temperature studies showed improved 11-OH-THC recovery in undiluted specimens (average + 13.7%) at 45 vs. 37°C. Decreased recovery (-55.9%) was observed at 55°C and 0% recovery at 65°C, presumably due to denaturing of the enzyme protein. Preliminary evaluation of the order of enzymatic and alkaline hydrolysis did not show substantial improvement in recovery of 11-OH-THC. THCCOOH concentrations were not substantially affected by enzyme source, amount of enzyme or temperature of hydrolysis. Due to the fact that THC concentrations were less than 10 ng/mL in all clinical specimens and were only present in a small number of specimens evaluated in this study, there are no conclusive findings on the affect of these changes on THC concentrations.

In agreement with the enzymatic hydrolysis procedure reported by Kemp *et al., E. coli* (β-glucuronidase enzyme is more effective than *H. pomatia* in hydrolyzing ether glucuronide linkages. Recovery was improved with an increase in temperature to 45°C. Less than 5000 U/mL *E. coli* β-glucuronidase substantially reduces the apparent concentration of 11-OH-THC; however, using 10,000 U/mL is prohibitively expensive. Another alternative would be to reduce the specimen volume, but this could result in decreasing THC concentrations below limits of quantification. These factors are important to consider when optimizing enzymatic hydrolysis during method development and validation.

Key Words: THC, Hydrolysis, Urine

P48

Evaluation of a Modified Emit® Immunoassay for Screening Fresh and Aged Whole Blood Specimens for the Detection of Marijuana Constituents

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Marijuana is the most commonly encountered drug in cases of "driving under the influence of drugs (DUID)" arrests. Numerous immunoassays for the detection of drugs in urine have been modified to detect drugs in fresh whole blood from DUID cases.

Objective: To evaluate the detection of marijuana metabolites in fresh whole blood and long stored refrigerated blood by a modified Emit marijuana metabolite immunoassay.

Methods: One hundred forty-five blood specimens submitted for DUID testing were initially analyzed by a modified Abbott TDx® marijuana metabolite fluorescence polarization immunoassay (FPIA) in an Abbott TDx® analyzer. Blood specimens were prepared by adding 400 µL of Abbott TDx® dilution buffer to 200 µL of sample, vortexing well, centrifuging and removal of the supernate which was pipetted into the analyzer for testing. The TDx® analyzer was calibrated with TDx® cannabinoid calibrators. All specimens were then tested by a modified Emit® marijuana metabolite immunoassay (Emit) in a Syva® 30R analyzer. Blood specimens for Emit testing were prepared by adding 2 mL of ice cold acetonitrile to 1.0 mL of whole blood while vortexing, centrifuging to obtain a supernate, then adding 100 µL of 0.1 M, pH 9.0 phosphate buffer which was reduced to ~200 µL in a Savant evaporator and transferred to the 30R analyzer. The 30R analyzer was calibrated with an extracts of drug free blood and a blood calibrator of 8 ng/mL of tetrahydrocannabinolic acid (THCA). Specimens were then analyzed for THCA by gas chromatography/mass spectrometry (GC/MS).

An additional 53 blood specimens, that had been stored refrigerated for at least one year and had previously tested negative for marijuana metabolites by FPIA when freshly collected, were reanalyzed using the modified Emit procedure described above. Again all specimens were analyzed by GC/MS for THCA.

Results: There was complete agreement of results between the FPIA and Emit in testing the 145 fresh blood specimens. Positive marijuana metabolite results were noted for 101 specimens tested by both modified immunoassays. All 101 of these specimens were confirmed THCA positive by GC/MS. No false positives or false negatives results were observed with either assay. However, with the long stored blood specimens that had tested negative by FPIA when freshly collected, all 53 specimens yielded positive results when tested by the modified Emit immunoassay. Only four of these specimens tested positive by GC/MS containing 7, 7, 8 and 12 ng/mL THCA.

Conclusion: Both modified FPIA and Emit immunoassays are equally efficient for the detection of marijuana metabolite in fresh whole blood. However, the Emit assay is unreliable for the detection of THCA in blood specimens after long-term refrigeration of greater than one year. The reason for consistent false positive results has yet to be determined. Therefore, if repeat testing for marijuana metabolite is requested in a long refrigerated blood specimens, GS/MS should be applied without initial Emit screening.

Key Words: Emit, Blood Marijuana, Immunoassay

P49

Comparison of Measurements of Carboxymyoglobin in Muscles in Relation to Carbon Monoxide in Blood

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Due to the lack of appropriate blood material in cases of fire accidents, other material often have to be taken into consideration for toxicology investigations. Muscle may be used as an alternative in forensic investigations of carbon monoxide detection.

Sample preparation of muscles with carboxymyoglobin was done according to a method where tissue is pulverized in a mill at low temperature. Carbon monoxide was measured by gas chromatography and hydrolysis to methane. Calculation of COHb saturation was based on the total iron method.

Samples from cases where low, medium and high levels of COHb saturation in blood were found, have been compared with the carboxymyoglobin in muscle from the same cases. A good correlation was found between levels at low concentration. At higher levels of saturation of the blood, it was not possible to compare the exact concentrations, although there seem to be a level of maximum carboxymyoglobin at 30 % saturation.

Key Words: Carboxymyoglobin, Carbon monoxide, Headspace-Gaschromatography

P50

Presence of Methamphetamine and Cocaine Pyrolytic Products in Light Bulb Trace Evidence and *ra«s-Phenylpropene as a Marker of Smoked Methamphetamine in Urine

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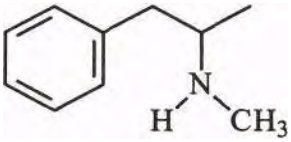
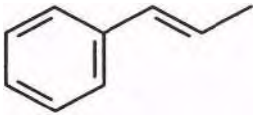
The object of this presentation is to present a case study that indicates that frans-phenylpropene is a marker for smoked methamphetamine and further characterizes cocaine and methamphetamine combinations as a function of mode of ingestion (smoked or other).

A 31 -year old male with a history of drug abuse was found dead as the result of an apparent drug overdose. Drug paraphernalia found at the scene included a syringe, straw and two hollowed out light bulbs. Several drugs of abuse were identified in blood and urine by GC/MS.

GC/MS and/or multistage MS analyses from the hexane eluants of the light bulbs confirmed the presence of methamphetamine, cocaine, /raws-phenylpropene, phenylacetone and anhydroecgonine methyl ester (AEME). trans-phenylpropene and phenylacetone are pyrolytic products of smoked methamphetamine (SekineH, *et al. J Forensic Sci.* 1990;35:580-590, Shakleya D, et al. *J Anal Toxicol,* in press) and AEME is derived from smoked cocaine (Jacob P 3rd, et al. *JAnal Toxicol.* 2004;5:141-150).





methamphetamine trans-phenylpropene

Drugs identified in blood included fentanyl (13 ng/mL), norfentanyl (3.8 ng/mL), methamphetamine (0.11 mg/L), amphetamine (0.09 mg/L), and benzoylecgonine (0.51 mg/L). Drugs and metabolites identified in urine included methamphetamine, amphetamine, cocaine, ecgonine methyl ester, fentanyl, oxycodone, acetaminophen, chlorpheniramine, and caffeine.

The characteristic pyrolytic products of cocaine, methamphetamine, and combinations of the two were evaluated using pyrolysis GC/MS. Preliminary investigations showed the presence of ecgonine methyl ester, N-formylmethamphetamine, methamphetamine, and substituted benzenes; the relative abundance of each was temperature dependent.

We conclude that detection of trans-phenylpropene in urine is the first example of using this compound as a marker of smoked methamphetamine use. Pyrolysis GC/MS analyses indicate that additional thermolytic breakdown products are present in the residue of smoked methamphetamine and cocaine mixtures.

Key Words: Cocaine, Methamphetamine, Pyrolytic products, *trans-Phenylpropene,* Marker

P51

The Analysis of Benzodiazepines in Urine using Solid Phase Extraction and LC/MS

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Benzodiazepines are widely prescribed drugs possessing anxiolytic, sedative hypnotic, anticonvulsant, and muscle relaxant properties. As a result of their widespread availability and CNS effects, Benzodiazepines are commonly abused, frequently in combination with alcohol or other drugs. Benzodiazepines are routinely monitored in urine specimens to detect abuse and monitor compliance. In the Emergency Department setting, Benzodiazepine analysis aids in the evaluation of patients with unknown causes of CNS depression. Our objective was to develop a sensitive and specific Liquid Chromatography/Mass Spectrometry (LC/MS) assay to confirm and quantify, following initial screening by immunoassay, the most commonly encountered Benzodiazepines.

Here we present a method that allows for the simultaneous determination of Alprazolam, Nordiazepam, Oxazepam, Temazepam, Lorazepam and Alpha-hydroxyalprazolam. This method utilizes Solid Phase Extraction (SPE) of the urine specimen, followed by Liquid Chromatography/Electrospray lonization/Mass Spectrometry. Sample, control, or calibrator (1 mL) is combined with Nitrazepam, used as the Internal Standard. Hydrolysis is accomplished by adding β-glucuronidase followed by incubation at 37°C for 90 minutes. Extraction was performed using a Cerex Polychrom SPE column with a positive pressure manifold (SPEWare Corporation). The column was washed and dried, followed by elution of drugs using 2% Ammonium Hydroxide in Ethyl Acetate. The samples were evaporated to dryness, reconstituted with Acetonitrile, and transferred to autosampler vials. Separation was achieved using an Atlantis dC_{18} 3.0X150mm (3.5µm particle size) column. The mobile phase consisted of deionized water/Ammonium Acetate/Acetonitrile, and the flow rate was 0.5 mL/min. The six Benzodiazepines eluted with retention times of 2 to 6 minutes. All compounds were identified using selected ion monitoring.

Performance characteristics were assessed using specimens prepared in-house from synthetic drug-free urine spiked with drug standard material. The lower limit of detection for all compounds was 50ng/mL. The lower limit of quantitation was 75ng/mL. The method is linear from 50ng/mL to l000ng/mL. Total (between-run) precision averaged 14.6%CV, and within-run precision averaged 2.3%CV. Accuracy, or trueness, averaged 98.6%.

This LC/MS method allows for identification and quantification of Benzodiazepines in a urine matrix. The method provides a high degree of sensitivity for commonly encountered Benzodiazepines, and offers adequate performance characteristics for routine clinical and forensic use.

Key Words: Benzodiazepines, LC/MS, Solid phase extraction

P52

Cocaine Analysis on a Bonded Pentafluorophenylpropyl High Performance Liquid Chromatography Stationary Phase Using Mass Spectrometry

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When cocaine is introduced into the body, several main metabolites are produced: benzoylecgonine, ecgonine and ecognine methyl ester. Urinalysis screening is performed and positive samples are submitted for confirmational analysis. Cocaine and its metabolites are traditionally analyzed and confirmed using GC/MS. Even though GC/MS methods are well established and do provide excellent confirmational data, they can be time consuming due to long analysis times and multiple sample preparation steps, which include derivatization. HPLC coupled with mass spectrometry provides an alternate chromatographic confirmation method for analyzing cocaine and its metabolites. Chemical standards of cocaine and its metabolites were prepared at various concentrations. HPLC methods were then evaluated for identification and quantification of each compound. Methods focused on choosing the best HPLC column stationary phase for maximizing sensitivity of all compounds while minimizing the total analysis time. This work demonstrates that the bonded pentafluorophenylpropyl stationary phase produces excellent sensitivity, symmetric peak shape and shorter analysis time when compared to traditional bonded CIS columns.

Key Words: Cocaine, HPLC, Mass Spectrometry

P53

Identification of Polydrug Use by Bilateral (Paired) Oral Fluid Specimen Collection

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Aims: A growing number of organizations throughout the world are adopting the use of oral fluid drug sampling and analysis as part of an appropriate workplace, clinical rehabilitation or criminal justice policy. The use of oral fluid testing is thought to offer a number of advantages when compared to urine sample collection and analysis. It is important in the case of any potential legal challenge to be able to offer a valid and robust method of oral sample collection that will allow split samples to be analyzed independently when necessary. The objective of this study was to present data for bilateral oral specimen collection following the laboratory analysis of such specimens.

Methods: A total of 124 paired oral fluid specimens were collected with prior consent from individuals undergoing rehabilitation treatment for drug dependency in five locations throughout the UK using the Intercept® DOA oral specimen collection device (OraSure Technologies, USA). A bilateral specimen collection technique was used which involved placing a separate collection device simultaneously on either side of the donor's mouth and leaving in situ inside the mouth for a total of two minutes. Specimens were then removed from the mouth and placed in a separate specimen vial that includes a preservative solution and sent for laboratory analysis using appropriate transport materials. There is an average three-fold specimen dilution when using the preservative. All specimens were collected and analyzed within a 4-week period. Each set of paired specimens were uniquely identified and initially screened for the presence of Opiates, Cocaine, Amphetamines and human IgG as a specimen validity measure, using separate OraSure Technologies Inc. Intercept® micro-plate enzyme immunoassays (cut-off calibrator concentrations (ng/mL) used for diluted specimens were 10, 5, 100 and 500 respectively). Using a second aliquot of each specimen found to be positive by immunoassay for any of the above drugs, confirmation of these drugs and a number of additional analytes not initially identified by immunoassay was performed by GC-MS-MS using previously validated cut-offs and published methods. The confirmation cut-off levels (ng/mL) were: Amphetamine (5), Cocaine (5), Benzoylecgonine (1.5), Morphine (1.0), Heroin (5), 6-AM (1.0), Codeine (0.5), Methadone (5), A-9-THC (0.25), and MDMA (5).

Results: All sets of bilateral specimens were of satisfactory quality following human IgG validity analysis. A total of 80 (65%) of the paired specimens were initially positive by immunoassay for a combination of opiates, cocaine or amphetamines. There were a total of 4 (3%) discrepant results between paired specimens by immunoassay, all of which were for the cocaine immunoassay. These paired specimens had a mean concentration difference of 0.4 ng/ml of benzoylecgonine. For each analyte listed, the number of bilateral specimens with detectable concentrations by GC-MS-MS, the range of concentration values (ng/mL) and the coefficient of determination of the concentrations (r^2) between the paired specimens were as follows: Amphetamine (13, 7.8-2700, 0.99), Cocaine (14, 6-1800, 0.96), Benzoylecgonine (33, 0.5-844, 0.73), Morphine (68, 1-1690, 0.91), Heroin (9, 7-710, 0.67), 6-AM (63, 1.3-5240, 0.97), Codeine (60, 0.7-192, 0.91), Methadone (40, 11-14000, 0.98), Δ-9-THC (16, 0.4-119, 0.91), and MDMA (3, 2.5-57, 0.99).

Conclusions: Bilateral oral fluid sampling and laboratory analysis offers a convenient, reliable and consistent method to determine the presence of the most commonly abused substances. The use of bilateral specimen collection using the Intercept® oral specimen collection device offers an alternative means to provide split specimens where required for legal challenges and in accordance with policy guidelines. A bilaterally collected set of oral specimens can be considered suitable to allow for independent verification of the confirmatory results identified with either specimen.

Key Words: Oral fluid, Bilateral, Drugs of abuse

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Amphetamine Excretion Following Tablet and Gel Formulations of Dexedrine

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Dexedrine (dextroamphetamine) is the dextro isomer of the compound d,l-amphetamine sulfate. Dexedrine was initially developed in the 1920's to treat depression and obesity. Soon after it was shown to be effective in the treatment of narcolepsy and attention deficit disorder with hyperactivity (ADHD). It is often prescribed to patients that do not respond well to Ritalin. Use of stimulant medications for the treatment of ADHD has increased dramatically in the last few years, not only in children and teenagers, but in the adult population as well. Because of their stimulant properties, amphetamines have a high potential for abuse. Often, these types of drugs are illegally sold or distributed for nontherapeutic purposes, making it important to establish the excretion profile of these drugs. Evaluation of urine and plasma concentrations of amphetamine following administration of Dexedrine in tablet form has previously been reported. In instances where a tablet cannot be used, a liquid/gel formulation of the drug can be prepared. Using a crossover design, subjects received either the tablet form or the gel form. Currently there is no data available describing urine and plasma profiles following administration of a typical therapeutic dose of Dexedrine in gel form. The data presented here describes the excretion profiles for both the tablet and the gel formulations of Dexedrine.

Subjects (n= 4) received a single 10 mg dose of Dexedrine in either the tablet formulation or a gel suspension. Blood samples were collected in lithium heparin tubes prior to administration of the drug and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 24, 36 and 48 hours following drug administration. Plasma was then separated from the sample and stored at < 20°C prior to analysis. Urine samples were collected in standard urine containers and stored at < 20°C prior to analysis. Samples were collected ad lib from each of the subjects prior to administration of the drug and at each urination for five days following initiation of the study.

Samples were analyzed using GC-MS following extraction of the analytes and derivatization with heptafluorobutyric anhydride. Plasma samples were extracted using solid phase extraction of a 1 mL aliquot with United Chemical Technologies XTRACKT, XRDAH203 high-flow 200 mg columns using a Zymark RapidTrace. Urine samples were extracted using liquid-liquid extraction of 2 mL sample aliquots.

Urine samples were positive (> 500 ng/mL) for no more than 48 hours following administration of either the tablet or gel form of the drug. The peak concentration of amphetamine seen in urine for the tablet form was 6,373 ng/mL and 2,722 in the gel form. Plasma samples for both formulations showed a peak concentration of 30 ng/mL and none contained detectable amphetamine (LOD 4 ng/mL) at 48 hours post dose. Amphetamine was detectable (LOD 5 ng/mL) in the urine of subjects administered the tablet form for up to 118 hours post dose and for up to 114 hours in urine samples after administration of the gel formulation.

Key Words: Dexedrine, amphetamine

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Cocaine and Metabolites in Urine After Controlled Smoked, Intravenous, Intranasal and Oral Cocaine Administration

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Cocaine is rapidly and extensively metabolized in humans. The appearance of cocaine's pharmacological activity varies with the route of administration, but the metabolic profiles following different routes are not substantially dissimilar. The objective of this project was to characterize urinary excretion profiles of cocaine and metabolites following controlled administration of cocaine by oral, intravenous (IV), intranasal (IN), and smoked (SM) routes. The NIDA Institutional Review Board approved two controlled cocaine clinical studies and participants provided informed consent, and resided on a closed, in-patient research ward under continuous medical surveillance. In study 1, six healthy males were administered cocaine by IV (25 mg), SM (42 mg), and IN (32 mg) routes. In study 2, six healthy males were administered cocaine by IV (0.0, 11.2, 22.4, and 44.8 mg), SM (0.0, 10, 20, and 40 mg), and oral (22.4 mg) routes. Specimens were collected up to six days after cocaine administration and analyzed by GC/MS for cocaine (COC), benzoylecgonine (BZ), methylecgonine (MEC), p-hydroxybenzoylecgonine (pHBZ), m-hydroxybenzoylecgonine (mHBZ), benzoylnorecgonine (NBZ), and ecgonine (EC) concentrations by a previously published method (Paul, Biomed Chromatogr, Epub 2005, April 19). Detection times and concentration ranges for each route (all doses) and each analyte are described below:

	Smoked	Intravenous	Intranasal	Oral
	Detection Times (h) Concentrations (ng/mL)	Detection Times (h) Concentrations (ng/mL)	Detection Times (h) Concentrations (ng/mL)	Detection Times (h) Concentrations (ng/mL)
BZ	1.4-106.3 21-22,494	1.2-74.5 21-20,460	0.8 - 70.6 21 - 23,403	2.2 - 67.8 21 - 9,949
COC	1.7-55.3 11 - 9,748	1.2-41.5 11-4,771	1.3-36.3 13-772	2.2 - 57.3 12 - 695
mHBZ	1.7-55.3 26 - 260	1.4-38.3 26-451	2.5 - 28.5 28 - 766	2.2-33.6 27 - 202
pHBZ	1.7-55.3 26-1,197	1.4-38.3 26-1,906	2.5-21.0 39-1,150	2.2-25.2 27 - 803
MEC	1.7-164.3 11-8,969	1.2-85.5 12-13,360	1.3-73.2 12-9,754	2.2-116.2 11 - 8,292
NBZ	1.8-32.4 26-3,592	1.4-38.3 26 - 960	2.5-21.0 34 - 768	2.2-30.1 27 - 956
EC	1.7-80.3 51-1,053	2.2 - 98.4 51-658	2.5-69.0 54 - 671	2.2 - 67.8 51 -457

Our study characterizes the disposition of cocaine and metabolites in urine following four routes of cocaine administration.

Key Words: Cocaine, Metabolites, Urine, GC/MS, Routes of administration

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Development of a Homogeneous Enzyme Immunoassay for the Detection of Ethyl Glucuronide in Urine and its Evaluation on the MGC 240 Analyzer

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The objective of the study was to develop a homogeneous enzyme immunoassay for the detection of Ethyl Glucuronide (ethyl -glucuronide, EtG) in human urine. The assay was developed on the Hitachi 917 analyzer and was evaluated on the new bench top automated analyzer MGC 240. The MGC 240 analyzer is a bench top automated analyzer with a throughput of 240 samples per hour.

Ethyl glucuronide is a direct metabolite of ethanol, which is formed by enzymatic conjugation of ethanol with glucuronic acid. Ethyl Glucuronide is stable, non-volatile, and water-soluble, and is an excellent biomarker for determining recent alcohol use and chronic alcoholism. Alcohol in urine is normally detected for only a few hours, whereas EtG can be detected up to 5 days. Therefore, EtG can be a useful diagnostic biomarker for monitoring abstinence in alcoholics in alcohol withdrawal treatment programs. EtG is a very specific biomarker of alcohol because unlike other biomarkers, EtG levels are not influenced by age, gender, and non-alcohol related diseases. Ethanol can be produced *in vitro* due to fermentation of urine samples containing sugars (diabetes), bacteria or yeast when samples are exposed to warm temperatures. In such cases, EtG test can be used, as a confirmatory test to determine if the alcohol in the sample is due to consumption of alcohol or it is formed *in vitro* as a result of fermentation. Currently EtG is monitored by GC/MS, LC/MS and LC/MS/MS.

Microgenics DRI® Ethyl Glucuronide Assay uses a monoclonal antibody that is highly specific to Ethyl Glucuronide. The assay is based on the competition between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug from the urine sample for a fixed amount of antibody binding sites. In the absence of free drug from the sample, the antibody binds the enzyme-labeled drug, causing a decrease in enzyme activity. Active enzyme converts NAD to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340 nm. The phenomenon creates a direct correlation between drug concentration in sample and enzyme activity.

The assay consists of two reagents-antibody/substrate reagent and enzyme conjugate reagent. The calibrators range from 0.0-50.0 mg/L. Reagents and calibrators are liquid ready-to-use. The following data were collected on the MGC240 analyzer. The within-run and between-run precision for calibrator levels 5.0-50.0 mg/L ranged from 3.2%-11.8%. The limit of detection (LOD) was 0.35 mg/L. No significant interference was observed from endogenous substances. The assay demonstrated very low cross-reactivity to other glucuronides. Method comparison studies on samples ranging from 2.0-500 mg/L were carried out on the Hitachi 917 and MGC 240 Analyzers using LC/MS/MS as reference method. Comparison of the immunoassay results with LC/MS/MS reference method yielded the following Deming's Regression: LC/MS/MS Vs. Hitachi 917: $y=0.99x-3$, $r=0.994$, $n=36$ and LC/MS/MS Vs. MGC 240: $y=0.95x+3$, $r=0.964$, $n=39$. Comparison of results obtained on the Hitachi 917 and MGC240, using 2 mg/L as cutoff, showed > 90% agreement with LC/MS/MS.

DRI Ethyl Glucuronide Assay performance on the MGC 240 is equivalent to Hitachi 917 analyzer. EtG Assay is a simple and convenient test for the detection of EtG in urine. The assay can be applied to several high throughput automated clinical chemistry analyzers.

Key Words: Ethyl Glucuronide, Enzyme Immunoassay, G6PDH

P57

A Micromethod For The Determination Of Blood With Matrix Modification By Graphite Furnace Atomic Absorption Utilizing A Graphite Tube

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The effect of low concentrations of lead on pre- and post natal growth and development is a growing concern. Several hundred thousand children, most of them living in older houses, are at risk of ingesting lead-based paint, as well as lead bearing soil and house dust contaminated by the deterioration of lead-based paint. We have developed a micro method utilizing 40 micro liters of whole blood. Graphite furnace technology coupled with the use of a matrix modifier has enabled the determination of lead in whole blood using aqueous standards for creating the calibration curve. The matrix modifier is prepared with 0.2% Triton X-100, 0.5% Ammonium Phosphate Dibasic, and 0.2% Nitric Acid. The standards are prepared in 1% Nitric Acid and diluted 1:10 with matrix modifier. The aqueous standards are compared to the NIST Standard Reference Material 955a and the values are adjusted accordingly. The Controls and patient samples are prepared by diluting 40 micro liters of whole blood with 360 micro liters of matrix modifier. The sample is dried in two steps (120° and 250° C), charred at 615° C, and atomized at 1700° C. Whole blood controls are obtained from BioRad. The sensitivity of the method is 1 ug/dL. The recovery of whole blood spiked with 10 ug/dL of lead standards is 101%. The quality control data and recovery studies indicate that this method is very good for the determination of lead in whole blood.

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The Analysis of Glucocorticoids by HPLC-MS (Quadrupole) & HPLC-MS (Time of Flight)

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The World Anti-Doping Agency (WADA) has developed regulations setting an international standard for drug testing in competitive sports. The 2005 prohibited drug list includes all forms of glucocorticosteroids. High-pressure liquid chromatography-mass spectrometry (HPLC-MS) provides (an effective alternative for the separation and analysis of compounds such as the glucocorticoids, that exhibit poor gas chromatographic characteristics. This paper presents a comparison of an Agilent 1100 series liquid chromatograph/mass selective detector (LC-MSD) using quadrupole technology and an Agilent 1100 series liquid chromatograph/mass selective detector (LC-MSD TOP) using time of flight mass spectrometry for the analysis of glucocortiocoids in urine. Atmospheric pressure chemical ionization was employed. Certified negative urine was fortified with eleven glucocorticoids; prednisone, prednisolone, methylprednisolone, betamethasone, dexamethasone, beclomethasone, flumethasone, desonide, flunisolide, triamcinolone acetonide, and budesonide at the WADA minimum required performance level (MRPL) of 30ng/mL. Samples were extracted using a previously published liquid-liquid extraction technique (Gotzman, et al. *Recent Advances in Doping Analysis* (12), 2004) using methyltestosterone as the internal standard at a concentration of 50ng/mL in urine. The LC column used was a Zorbax Eclipse XDB-C18, 5|Lim, narrow-bore 2.1x150mm (Agilent). Mobile phases consisted of 0.1% formic acid solution in MilliQ water and acetonitrile. Chromatographic separation with baseline resolution of all eleven glucocorticoids was achieved using a mobile phase gradient from 28% to 90% acetonitrile with a flow rate of 0.3 mL/minute, over an 18 minute (analysis) time. Compounds with identical mass to charge ratio such as triamcinolone acetonide and flunisolide were effectively separated chromatographically. The betamethasone and dexamethasone stereoisomers were well resolved as was an epimer mix of budesonide. The eleven glucocortiocoids were chromatographically separated and identified in urine extracts at the WADA minimum required performance level of 30 ng/mL using both quadrupole and time of flight detection.

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KeyWords: LC-MS, Glucocorticoids, Time of Flight

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A Case of Nearly Uniform Post-Mortem Redistribution

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Postmortem Redistribution (PMR) of drugs is one of the confounding factors limiting the toxicological significance of drug concentrations in forensic investigations of deaths. Understanding which drugs are most likely to undergo PMR assists with toxicological interpretations of findings, especially when peripheral samples of blood may be limited. Amongst the factors that correlate with PMR is volume of distribution (Vd) of a drug; the greater the Vd the more likely a drug will undergo PMR. This case presents a marked deviation from the correlation with Vd as all of the detected drugs that were quantified demonstrated a fairly uniform PMR, but have different Vds. This case involves a 51 yr/old female, past drug abuser and heavy drinker with a history of psychiatric disorder. She was feeling unwell and in the company of a friend when it was noticed that she had stopped breathing. Despite resuscitative attempts at the scene, she was pronounced dead in hospital approximately 1-1/2 hours later. An autopsy was carried out approximately 15 hours after death with no remarkable findings and toxicology was ordered.

Our laboratory routinely screens heart blood and quantifies most drugs in femoral blood samples in order to make effective use of submitted samples and reduce the possibility of over-estimation of drug quantities due to PMR. Our screening procedure includes a GC/MS basic drug screen that, at the time of this investigation, was also used to quantify 12 drugs common to a wide variety of investigations including, codeine, diazepam and nordiazepam, which were found in this case. This method also identified the presence of oxycodone, hydrocodone, perphenazine, paroxetine and benztropine. The GC/MS screening was performed on heart blood (HB) and confirmed by GC or GC/MS on femoral blood (FB).

There was a remarkable correlation between the redistribution of five of the drugs detected (limited blood samples prevented quantitation of all drugs in both heart and femoral blood) which collectively had a mean Heart:Femoral blood concentration ratio (HFR) of 2.04 (± 0.51). Codeine showed the lowest HFR at 1.37 while paroxetine displayed the highest HFR at 2.56. The data had a mean 95%CI value of 0.04. In comparison, the correlation to volume of distribution was poor. Comparing the HFR with Vd provided correlation coefficients (R^2) ranging from 0.1858 to 0.6434 when using both low and high ends of the Vd ranges for these drugs. The data do not wholly discount the effect of Vd on PMR of the drugs in this case, but invite the possibility of a common mechanism and/or source for the elevated HB drug concentrations relative to FB.

Key Words: Postmortem redistribution, case report, five drugs