AUTHOR AND KEYWORD

INDEX

INDEX BY AUTHOR

PRESENTING AUTHOR	ABSTRACT
Meatherall, Robert	30
Mertens Maxham, Diane	19
Miranda, Estuardo	13
Moody, David	28
Moore, Karla	16
Negrusz, Adam	25
Osselton, M.D.	22
Patel, Jayant	P27
Paul, Buddha	9
Peace, Michelle	21
Pearson, Julia	32
Poklis, Alphonse	10
Poklis, Justin	P22
Ropero-Miller, Jeri	12
Saady, Joseph	14
Sahin, Elvan	37
Salamone, Salvatore	39
Singer, Peter	17
Stout, Peter	6
Stout, Peter	35
Tilson, Christopher	29
Umberger, Logan	P32
Vasiliades, John	P13
Wagner, Michael	38
Whitehead, Robert	P11
Wiegand, Russell	7
Wilkins, Diana	P9
Wold, Carl	P30
Wong, Baguio	P24
Wong, Raphael	P33
Wong, Stephen	36
Wong, Stephen	P12
Wood, M	P19
Wood, Michelle	P25

INDEX BY KEY WORDS

ABSTRACTS

PLATFORM PRESENTATIONS

1

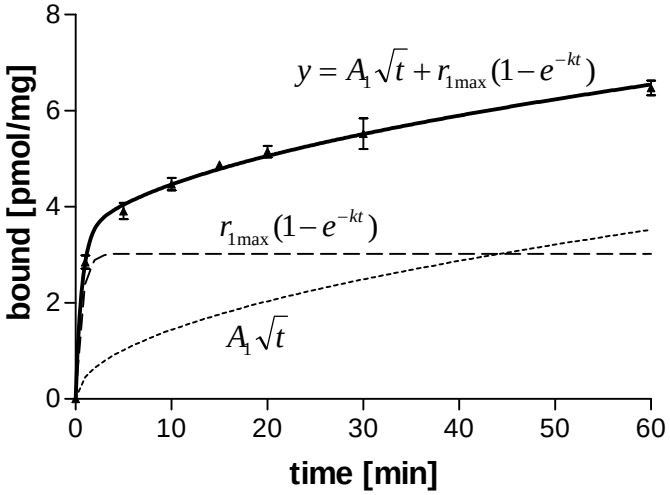
Characterization of [^{3}H]-Flunitrazepam Binding to Melanin

*Martin F Testorf[1], **Robert Kronstrand[4*]**, Samuel P.S. Svensson[2], Ingemar Lundström[3], and Johan Ahlner[4]. [1]Dept of Biomedical Engineering, [2]Division of Pharmacology, [3]Division of Applied Physics, Linköping University, 581 85 Linköping, Sweden, [4]National Board of Forensic Medicine, Department of Forensic Toxicology, University Hospital, 581 85 Linköping, Sweden*

In both clinical and forensic toxicology, the analysis of hair for drugs is an important tool to determine drug use in the past or to verify abstinence from illegal drugs during extended periods. Melanin is proposed as one of the factors that influence drug incorporation to hair and we have characterized the binding of the drug flunitrazepam to melanin *in vitro*.

Tritiated flunitrazepam and melanin granules from cuttle fish, *Sepia officinalis*, were used. Melanin was incubated with [^{3}H]-flunitrazepam at room temperature for 60 min in glass tubes with 250 µl PBS. During incubation the tubes were placed on a shaking rack (2 Hz). Separation of free drug from melanin was performed with a cell harvester (Brandell, U.K.) with GF/C filters (Whatman, Sweden). The filters were washed twice with 3 ml 50 mM potassium phosphate buffer, then placed in polyethylene scintillation vials and dissolved in 5 ml scintillation fluid during 45 min on the shaking rack. The radioactivity was then measured in a liquid scintillation counter (1217 Rackbeta, Wallac, Sweden). In the displacement experiments, melanin (0.04 mg/ml) was incubated for 60 min with 5 nM [^{3}H]-flunitrazepam and increasing concentrations of the unlabelled displacing drug.

The results showed a rapid binding followed by, not saturation, but an almost linear slope of slowly increasing drug binding. A solely electrostatic attraction to the surface would decrease the more drug that bind until saturation. The data fitted excellently to a curve composed of one term containing the square root of time added to one Langmuir binding term. See figure. Initially, the Langmuir dominating binding may reflect a superficial binding to the surface of the melanin granule. This is followed by a binding limited by diffusion as is suggested by the fit to the square root of time. We believe that this binding reflects the diffusion of drug molecules into the matrix of melanin deeper in the granule. We chose to call these *surface binding* and *bulk binding*, respectively.



Displacement experiments showed that the benzodiazepines oxazepam, diazepam, nordiazepam and nitrazepam all displace [^{3}H]-flunitrazepam in a very similar way to flunitrazepam itself indicating similar binding characteristics. The metabolite 7-amino-flunitrazepam, and the tranquillizers zopiclone and zolpidem also displace [^{3}H]-flunitrazepam but to a somewhat lower degree. Phenobarbital, which in contrast to the other drugs and metabolites investigated, is a weak acid had a significantly lower ability to displace [^{3}H]-flunitrazepam.

The method developed in this study allowed measurements with low melanin and drug concentrations, and had the strength to propose binding characteristics for flunitrazepam to melanin as well as measure the relative binding of several other tranquillizers.

This project was partly funded by research grant 47-311-6 from the National Board of Forensic Medicine.

Key words: Benzodiazepines, Melanin, Hair

2

Enantiomer Profile of Amphetamine and Methamphetamine Derived from Famprofazone

***Brandy Greenhill*[1]*, *Sandra Valtier*[2], *and John T. Cody*[3]. *[1]University of Texas Health Science Center at San Antonio TX, *[2]Clinical Research Squadron, Wilford Hall Medical Center, Lackland AFB, TX, *[3]Academy of Health Sciences, Ft. Sam Houston, TX*

There are several drugs that lead to the production of methamphetamine and/or amphetamine in the body that are subsequently excreted in the urine. These drugs raise serious concerns when interpreting positive amphetamine drug testing results. Analysis of the concentrations of the amphetamines and their enantiomeric profile can assist in determining whether the drug was from one of these precursor drugs or from the use of methamphetamine. One of these drugs is famprofazone (Gewodin, Geistlich) which is an over-the-counter analgesic not available in the United States, but is available in European countries, such as Germany.

Following Institutional Review Board approval, two Gewodin tablets (50 mg of famprofazone) were administered orally to five volunteers with no history of amphetamine, methamphetamine, or famprofazone use.The subject population consisted of 3 females and 2 males ranging in age 29-42 in good health. Following administration, urine samples were collected *ad lib* for up to seven days and the pH, specific gravity, and creatinine values were determined to assess variations in the measured concentrations of methamphetamine and amphetamine. To determine the enantiomeric profile of methamphetamine and amphetamine, samples were prepared using liquid-liquid extraction, derivatized with N-trifluoroacetyl-*l*-prolyl-chloride, and analyzed by gas chromatography/mass spectrometry (GC/MS) in electron ionization mode. Samples were injected on to a HP-1 (12m x 0.2mm i.d. and 0.3µm film thickness; Hewlett-Packard) column.

Analysis showed the famprofazone metabolites amphetamine and methamphetamine to be both *d*- and *l*-enantiomers. The proportion of *l*-methamphetamine exceeded that of its *d*-enantiomer from the first sample collected. In all subjects, the proportion was approximately 70% *l*-methamphetamine initially and this proportion increased over time. Amphetamine results showed *l*- and *d*-amphetamine were virtually the same in the early samples with the proportion of *l*-amphetamine increasing only slightly as time progressed.

Interpretation of results is a critical part of forensic drug testing due to potential repercussions to an individual. The finding of both enantiomers alone differentiates famprofazone use from the most commonly abused form of methamphetamine and all medicinal methamphetamine available in the U.S., which is either *d*-methamphetamine or *l*-methamphetamine (Vick's inhaler). Coupling this information with the concentrations of methamphetamine and amphetamine helps to determine the potential for use of this drug.

Key words: Famprofazone, Enantiomer

3

The Pharmacological Characterization of Gamma Valerolactone (GVL) in Rats as Compared to Gamma Hydroxybutyric Acid (GHB), Gamma Butyrolactone (GBL) and Ethanol (EtOH)

Laureen J. Marinetti* *and Randall L. Commissaris. Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, 259 Mack Avenue, Detroit, MI 48202*

The present studies compared the central nervous system depressant effects of GVL, GHB, GBL and EtOH. GVL is the gamma methyl substituted structural analog of GBL.Upon ingestion GVL is quickly metabolized by blood or liver lactonase enzyme to gamma methyl, gamma hydroxy butyric acid (methyl-GHB), Fishbein and Bessman 1966. In vitro receptor studies have been performed to access the binding affinity of methyl-GHB to the GHB receptor, Bourguignon et al. 2000. Although the binding affinity of methyl-GHB was less than with GHB at the 1 micromole concentration there was still a measurable amount of inhibition of [^{3}H]GHB binding, indicating that methyl-GHB does have some affinity for the GHB receptor. However no animal studies were conducted to access the pharmacological activity of either GVL or methyl-GHB.

GVL is currently being listed under the chemical synonym 4-pentanolide as the active ingredient in some new diet aid products being marketed on the internet as GHB/GBL replacements. Since GVL is not a structural analog of GHB nor are there studies confirming that GVL has pharmacological activity similar to GHB – it is not currently a controlled substance either by being listed or by definition as a controlled substances analog.

The acoustic startle paradigm was used to measure central nervous system depression in male Sprague Dawley rats. The startle is the “jump” associated with a brief noise burst and has been shown to be sensitive to sedative and hypnotic agents such as ethanol and GHB. The drug treatments were administered intraperitoneally with a 15 minute pretreatment and were as follows: Vehicle (distilled water), 100, 200 and 400 mg/kg GHB, 37.5, 75, and 150 mg/kg GBL, 500, 1000 and 2000 mg/kg EtOH and 200, 400 and 800 mg/kg GVL. The amplitude of the startle or jump was measured and was found to decrease as the drug dose was increased. A dose vs response curve was generated with the dose depicted on the x-axis and the amplitude of the startle response (percent of vehicle control) depicted on the y-axis. The order of potency on the acoustic startle response is as follows: GBL >> GHB > GVL >> EtOH. All treatments showed equal efficacy in their ability to depress the startle response to the same degree if enough of the compound was administered. In the acoustic startle paradigm GVL has pharmacological activity similar to GHB with a similar potency. GVL is not as potent as GBL but is much more potent than EtOH.

Key words: GHB, GVL, Central Nervous System Depression

4

An LC-APCI-MS/MS Method for Oral Fluid and Urine to Monitor Maternal Drug Use in a Clinical In-utero Drug Exposure Study

R. Dams[*1,2], *C. Murphy*[1], *R.. Choo*[1], *W. Lambert*[2], *and M. Huestis*[1]. [1]*National Institute on Drug Abuse, 5500 Nathan Shock Drive, Baltimore, MD, 21224, USA;* [2]*Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium*

Objectives: The objective of this work is to develop a quantitative LC-APCI-MS/MS method for the analysis of approximately 30 analytes, including opioids, cocaine, methadone and their metabolites. The method will be used to evaluate the suitability of oral fluid as an alternative to urine drug monitoring in pregnant opiate and cocaine addicts.

Methods: All experiments were carried out on an LCQ Deca XP Ion Trap Mass Spectrometer interfaced to a Surveyor HPLC system (ThermoFinnigan, CA). Chromatographic separation was performed on a Synergi Polar RP column (150 X 2.0 mm , 4µm, Phenomenex, CA), using gradient elution with (A) 10 mM ammonium formate in water, 0.001% formic acid (pH=4.5) and (B) acetonitrile, at a flow rate of 300µl/min. All samples were pretreated with acetonitrile protein precipitation and concentration of the organic phase.

Results: Separation of all analytes was accomplished in 25 minutes. Calibration curves were obtained using linear regression analysis, with a correlation coefficient (r^2) of greater than 0.99 for each of the analytes. LOQ were established in the low ng/mL range (1-15 ng/mL), except for morphine-6-glucuronide. Intra and inter-day reproducibility of the method proved to be less than 21.2 (%RSD) for all compounds. APCI was found to be a stable ionization technique, exhibiting minimal ion suppression, primarily in the first 2 minutes of the analysis. The method is being applied to a clinical study monitoring the effects of fetal exposure to drugs of abuse. This presentation focuses on maternal samples used to monitor drug use throughout pregnancy. Our main interest is in the suitability of oral fluid as an alternative matrix for drug testing. An advantage of oral fluid is the non-invasive character of sample collection. Samples are obtained without exposing patients to discomfort, skin irritation or risk of infection, and also provides less opportunity for sample adulteration. The primary form of the drug found in oral fluid is parent drug, permitting a direct comparison of pharmacologically active drug concentration to observed effects. However, a disadvantage to monitoring drug use with oral fluid testing is that the detection windows for active drugs may be shorter than those of drug metabolites in urine.

Conclusion: Protein precipitation combined with LC-APCI-MS/MS provides a fast, efficient method for the quantitation of a wide variety of illicit drugs and their metabolites in oral fluid and urine. LC-APCI-MS/MS enabled us to assess the usefulness of oral fluid as an alternative matrix for drug monitoring in an in-utero drug exposure study.

Key words**:** Oral fluid, In-utero, Liquid chromatography-tandem mass spectrometry

Acknowledgements: R.D. gratefully acknowledges the travel grant V 4.037.01N from the F.S.R.-Flanders (Belgium).

5

Pharmacogenomics as Molecular Autopsy for Forensic Toxicology: Genotyping Oxycodone Cases for Cytochrome P450 *2D6*

Paul J. Jannetto*[1,2], *Steven H. Wong*[1,2], *Susan B. Gock*[1,2], *Elvan Laleli-Sahin*[1,2], *B. Charles Schur*[2], *and Jeffrey M. Jentzen*[1,2]. *[1]Milwaukee County Medical Examiner's Office; [2]Department of Pathology, Medical College of Wisconsin, Milwaukee, WI, U.S.A.*

Pharmacogenomics, the study of the impact of heritable traits on pharmacology and toxicology, may serve as an adjunct for certifying opioid fatality. Oxycodone, a semi-synthetic opioid agonist with pharmacological actions similar to those of morphine, is frequently prescribed for the relief of moderate to severe pain. Several recent reports by the Drug Abuse Warning Network (DAWN) have shown that not only the use of oxycodone, but also the abuse of oxycodone is on the rise as indicated by the increased number of oxycodone mentions in the Emergency Department and Medical Examiners reports. Oxycodone is metabolized to oxymorphone by cytochrome P450 (CYP) 2D6. The gene encoding this enzyme is highly polymorphic with three mutations (**3*, **4*, and **5*) having a combined 95% allelic frequency. The prevalence of these three mutations in the general population is about 10%, making these individuals potentially more susceptible to oxycodone toxicity. By establishing the prevalence of *CYP2D6* polymorphisms and examining covariables, we hypothesized that oxycodone fatality may be partially due to poor drug metabolism caused by *CYP2D6* variant alleles.

From the Milwaukee County Medical Examiner's Office, a retrospective analysis of oxycodone cases including current cases from January 2000 to March 2002 was performed. Cases that included oxycodone as a contributing factor in the cause of death were genotyped to evaluate the use of pharmacogenomics as an adjunct to certify the cause and manner of death in forensic toxicology. Prior to the initiation of this study, Institutional Review Board approval was obtained. Oxycodone was extracted from blood and/or urine and identified by full scan electron impact GC/MS and quantified using selective-ion-monitoring. Blood samples were genotyped for *CYP 2D6 *3*, **4*, and **5* variant alleles by conventional and real-time PCRs.

The results show that two of the fifteen cases certified with oxycodone toxicity were homozygous for *2D6*4* and four were heterozygous for *2D6*4*. In twelve of the fifteen cases, the cause of death was identified as mixed drug toxicity while the remaining three were solely due to an overdose of oxycodone. Overall, the prevalence of *CYP2D6* variant alleles in cases at the Medical Examiner's Office that listed oxycodone in the cause of death was not significantly different from those in the control group (n = 26) (p >0.05, Fisher's Exact Test). However, genotyping *CYP2D6* did provide additional information that could possibly explain the oxycodone toxicity in four of the six cases that were shown to have decreased or completely impaired metabolism.

Although this study did not establish a direct relationship between the enzyme deficiencies associated with the *CYP2D6* variant genotype and the oxycodone fatalities, the genotyping results still provided a more definitive interpretation of the oxycodone toxicity. Therefore, these findings demonstrate the usefulness of pharmacogenomics as an adjunct in the determination of the cause and manner of death in forensic toxicology. Based on this experience, a pharmacogenomic algorithm for genotyping in forensic toxicology cases has been proposed.

Key words: Oxycodone, Pharmacogenomics, *CYP 2D6*.

6

Rapid Simultaneous Determination of Amphetamine (AMP), Methamphetamine, 3,4-Methylenedioxyamphetamine (MDA), 3,4-Methylenedioxymethamphetamine (MDMA) and 3,4-Methylenedioxyethylamphetamine (MDEA) in Urine by Solid Phase Extraction and GC/MS: A Method Optimized for High Volume Laboratories.

***Peter R. Stout**, Carl K. Horn and Kevin L. Klette. Navy Drug Screening Laboratory, PO Box 113, Bldg. H-2033, Naval Air Station, Jacksonville FL, 32212*

To facilitate analysis of high sample volumes, an extraction, derivitization and GC/MS analysis method was developed to simultaneously determine amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxyamphetamine (MDA) 3,4-methylenedioxy-methamphetamine (MDMA) and 3,4 methylenedioxyethylamphetamine (MDEA) in urine. This method utilized a positive pressure manifold cation-exchange polymer-based solid phase. The samples were poured into 3 ml columns and positive pressure (2-5 psi) was applied to the columns. Next, 1 ml of water was applied to the column followed by 1 ml 0.1M HCl and dried for 2 minutes at 25 psi. The column was then washed with 1 ml methanol and 1 ml ethyl acetate. Again the column was dried for 2 minutes at 25 psi. The target compounds were eluted into automated liquid sampler (ALS) vials with the addition 1.0 ml of ethyl acetate: methanol: ammonium hydroxide [80:20:2 v/v] to the column. Prior to drying, 0.050 ml of 1% HCl in methanol was added to the sample to reduce the potential to volatilize the target compounds. Samples were then evaporated to dryness under a stream of nitrogen and rapid derivitization was accomplished using heptafluorobutyric anhydride (HFBA).

Recoveries averaged 90% or greater for each of the compounds. Limits of detection were 62.5 ng/ml (AMP and MDEA), 15.6 ng/ml (MAMP), 31.3 ng/ml (MDA and MDMA) using a 2 ml sample volume. The method was linear to 5,000 ng/ml for all compounds using D5-MDMA and D14-MAMP as internal standards. Two hundred human urine samples were analyzed by this method. Excellent agreement was seen with previous quantitations as demonstrated by a paired two-tailed t-test ($p<0.05$). The method was challenged with 75 potentially interfering compounds and no interferences were seen. These interfering compounds included ephedrine, pseudoephedrine, phenylpropanolamine and phenethylamine. The method resulted in dramatic reductions in processing time (approximately 2 hours per batch) and waste production (elimination of chlorinated solvents).

Key words: Urine, Methamphetamine, Amphetamine, Ecstasy, GC/MS

7

Comparison of EMIT® II, CEDIA®, and DPC® RIA Assays for the Detection of Lysergic Acid Diethylamide in Forensic Urine Samples

Russell F. Wiegand*, *Kevin L. Klette, Peter R. Stout, and Jay M. Gehlhausen. Navy Drug Screening Laboratory, Naval Air Station, H-2033, Jacksonville, FL, 32212*

In an effort to determine a practical, efficient, and economical alternative for the use of a radioimmunoassay (RIA) in the detection of lysergic acid diethylamide (LSD) in human urine, the performance of two photometric immunoassays (Dade Behring EMIT® II and Microgenics CEDIA®) and the Diagnostics Products Corp. (DPC ®) RIA were compared. Precision, accuracy, and linearity of the three assays were determined by testing 60 replicates (10 for RIA) at five different concentrations below and above the 500 pg/mL LSD cutoff. The CEDIA and RIA exhibited better accuracy and precision as compared to the EMIT II immunoassay. In contrast, the EMIT II and CEDIA demonstrated superior linearity r^2 = 0.9809, 0.9540, respectively, as compared to the RIA (r^2 = 0.9062). The specificity of the three assays was assessed using compounds that have structural and chemical properties similar to LSD, common over-the-counter (OTC) products, prescription drugs and some of their metabolites, and other drugs of abuse. Of the 144 compounds studied, the EMIT II cross-reacted with eight compounds whereas the CEDIA and RIA only cross-reacted with four of these. Specificity was also assessed in 221 forensic human urine specimens that previously screened positive by the EMIT II assay. Of these, only 11 tested positive by CEDIA, and 3 by RIA. This indicated that the RIA was the most specific of the three tests. Of these 221 EMIT positives, only those also positive by RIA were analyzed by gas chromatography-mass spectrometry (GC-MS). None of the samples were positive at a cutoff concentration of 200 pg/mL. Each of the IAs correctly identified LSD in twenty-three out of twenty-four human urine specimens that had previously been found to contain LSD by GC-MS at the cutoff concentration. The CEDIA exhibited superior precision, accuracy, and decreased cross reactivity to compounds other than LSD as compared to the EMIT II assay and does not necessitate the handling of radioactive materials.

Key words: LSD, Immunoassay, Urine

8

Comparison of LC/MS Ionization Techniques for Cannabinoid Analysis in Blood

John M. Hughes*[1], *David M. Andrenyak[2], Dennis J. Crouch[2], and Matthew Slawson[2]. [1]Agilent Technologies, Pleasanton, CA; [2]Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT*

THC and its major metabolites have been analyzed by GC/MS using both electron impact and chemical ionization techniques. Though in wide use, these techniques require extensive sample preparation and derivatization. The availability of routine and affordable benchtop LC/MS systems has allowed the development of sensitive and robust methods for the analysis of drugs of abuse which can use existing sample preparation methods but do not require derivatization steps. The objective of this study was to develop a method using API-LC/MS capable of quantitating THC and 11-OH-THC at 1 ng/mL and THC-COOH at 10 ng/mL in plasma, in a single analysis. The quantitation objective was targeted for 1mL of blood, the extract reconstituted to 100µL and an injection of 20 µL.

Three API modes (electrospray ionization [ESI], atmospheric pressure chemical ionization [APCI], and the newest mode, atmospheric pressure photoionization [APPI] were evaluated for the analysis of THC, 11-hydroxy-THC and carboxy-THC, initailly using unextracted standards. APCI provided better sensitivity than ESI for all three analytes, especially using time-programmed positive/negative ionization switching to take advantage of the ionization preferences of the analytes (11-OH-THC and THC-COOH having better sensitivity in negative ion mode, THC more sensitive in positive ion). APPI was more sensitive than APCI for all three analytes by a factor of 3-5x. APCI also would not reach the 1ng/mL LOQ for THC in actual extracts. APPI was therefore utilized to evaluate two SPE procedures for sample preparation.

Three HPLC columns and several HPLC mobile phases were evaluated for speed, chroamtography, and efficacy in separating the considerable interferences found in blood extracts. All columns were 2.1 mm i.d., 150 mm in length, contained 5u particles, and were used at a flow rate of 400 µL/min. The Zorbax StableBond CN column, chosen to be quite different from more non-polar stationary phases, was found to give inadequate separation of 11-OH-THC and THC-COOH using all three mobile phase systems described below. Zorbax Eclipse XDB-C8 and Zorbax StableBond Phenyl both gave good separation and peak shape, and the latter was determined to give superior sensitivity with the acetone-based mobile phase found to be optimal for APPI.

Mobile phase systems of acetonitrile/water (with and without ammonium acetate modifier), MeOH/ammonium acetate, and acetone/ammonium acetate gave good separation and peak shape on the XDB-C8 and SB-Phenyl columns. Ammonium acetate at 50mM was necessary for this mobile phase for the detection of all three compounds in ESI negative ion mode, and the MeOH system gave significantly better sensitivity in APCI than the ACN system. A gradient of MeOH/5mM ammonium acetate (50-90% MeOH over 8 minutes) or acetone/5mM ammonium acetate (50-85% B over 8 minutes) gave both good chromato-graphic separation and good sensitivity when used with APPI (adding acetone dopant post-column with the MeOH system). The acetone-based mobile phase gave better sensitivity than MeOH with post-column addition of dopant.

(continued)

SPE methods using Bond Elut Certify (Varian, Inc) and Cerex Polychrom-THC (Cera, Inc.) columns were evaluated, using a previously published procedure (JAT 25, 531-537, 2001) and the manufacturer's procedure, respectively. Both procedures utilized acetonitrile deproteination followed by loading of sample in acetate buffer, and a final elution comprised primarily of ethyl acetate/hexane, with the final extract reconstituted with 100 μL of 1:1 acetone/water with 0.1% formic acid. The Cerex polymer column provided noticeably cleaner extracts, although there are still interferences with 11-OH-THC in some samples. Additional work is needed to improve the cleanliness of the extracts and the recovery of THC (currently only 30%).

The current APPI method uses an injection volume of 20μL, a vaporizer temperature of 400°C, SIM ions of 315 (THC positive ion), 329 and 343 (11-OH-THC and THC-COOH respectively, both in negative ion mode), capillary voltage 2500V positive and 3500V negative, and fragmentor voltage 175V.

Key words: THC, Blood, LC/MS

9

Effects of Oxidizing Adulterants on Detection of 11-Nor-Delta-9-THC-9-Carboxylic Acid in Urine

Buddha D. Paul* *and Aaron Jacobs. Division of Forensic Toxicology, Office of the Armed Forces Medical Examiner, Armed Forces Institute of Pathology, Rockville, Maryland 20850, U.S.A.*

Background: Bleach, nitrite, chromate, and hydrogen peroxide-peroxidase are effective urine adulterants used by the illicit drug users to conceal marijuana-positive results. Methods for detecting nitrite and chromate are available. Effects of other oxidizing agents that could possibly be used as adulterants and are difficult to detect or measure are presented in this report.

Methods: Urine samples containing 40 ng/mL of 11-nor-delta-9-THC-9- carboxylic acid (THC-acid) were treated with 10 mmol/L of commonly available oxidizing agents. Effects of horseradish peroxidase of activity 10 unit/mL and extracts from 2.5 g of red radish (*Raphanus sativus*, Radicula group), horseradish (*Armoracia rusticana*), Japanese radish (*Raphanus sativus*, Daikon group), and black mustard seeds (*Brassica nigra*), all with 10 mmol/L of hydrogen peroxide, were also examined. After 5 min, 16h and 48h of exposure at room temperature (23^0C) the specimens were tested by a gas chromatography-mass spectrometric method for THC-acid. A control group treated with sodium hydrosulfite to reduce the oxidants, was also tested to investigate the effect of oxidizing agents on THC-acid in the extraction method.

Results: THC-acid was lost completely in the extraction method when treated with chromate, nitrite, oxone®, and hydrogen peroxide/ferrous ammonium sulfate (Fenton's reagent). Some losses were also observed with persulfate and periodate (up to 25%). These oxidants, and other oxidizing agents like permanganate, periodate, peroxidase, and extracts from red radish, horseradish, Japanese radish and black mustard seeds destroyed most of the THC-acid (>94%) within 48h of exposure. Chlorate, perchlorate, iodate, and oxychloride under these conditions showed little or no effect. Complete loss was observed when THC-acid was exposed to 50 mmol/L of oxychloride for 48 h.

Conclusion: Several oxidizing adulterants that are difficult to test by the present urine adulterant testing methods showed considerable effects on the destruction of THC-acid. The time and temperature for these effects were similar to that used by most laboratories to collect and test specimens. In several cases the loss of THC-acid was >94%.

Key words*:* Oxidizing adulterants, Urine test, THC-metabolite

10

A Case of Homicide by Chronic Arsenic Poisoning with Apparent Radiographic Evidence of Arsenic Administration

Alphonse Poklis. *Department of Pathology, Medical College of Virginia Campus at Virginia Commonwealth University, Richmond, VA 23298-0165*

A case of homicide by chronic arsenic poisoning is presented. The victim was a 30 yr. mother of two, who was in excellent health until eight months prior to her death when she developed an apparent viral syndrome characterized by persistent nausea, vomiting and diarrhea with low grade fever and a rash. Within 2 weeks she developed a symmetrical paresthesia and weakness in both her hands and feet with difficulty in walking.These symptoms progressed and resulted in a diagnosis of Guillian-Barre syndrome. During the following two months she had several episodes of severe gastrointestinal distress with persistent vomiting and diarrhea. Two weeks prior to her death she was hospitalized for mental confusion, hypotension and life threatening seizures. Her condition improved until she had dinner with her husband, after which she developed severe gastrointestinal distress. Her condition continually deteriorated until her death from apparent ARDS and sepsis. Arsenic poisoning was ruled the cause of death after a misplaced clinical toxicology test result was discovered shortly after her death. Blood collected two weeks prior to her death revealed arsenic concentration of 21 mg/L.A urine specimen collected two days prior to death contained 188 mg/L arsenic and postmortem liver contained 170 mg/Kg. Analysis of these specimens was by atomic absorption spectrophotometry. Sequential hair analysis for arsenic by neutron activation demonstrated that the victim had been poisoning for at least eight months prior to her death. X-rays taken shortly after the victim ate dinner with her husband disclosed radiopaque material in the stomach consistent with arsenic trioxide. The victim's husband had purchased arsenic trioxide through his work. Evidence was amassed against the husband who admitted to murdering his wife by chronic arsenic poisoning.

Key words: Homicide; Arsenic poisoning; Radiography

11

Postmortem Tissue Concentrations of Nefazodone

Kabrena E. Goeringer* *and Olaf H. Drummer. Victorian Institute of Forensic Medicine, Department of Forensic Medicine, Monash University, Southbank 3006, Australia*

Nefazodone (Serzone®, Bristol-Myers Squibb), is an atypical antidepressant structurally unrelated to the selective serotonin reuptake inhibitors (SSRIs). It is often referred to as a serotonin antagonist reuptake inhibitor, which combines a powerful antagonism of serotonin 5-HT_{2A} receptors and some noradrenaline reuptake blockade. Like the SSRIs, its maximum antidepressant effects take several weeks to be realized. Formation of the major metabolites, hydroxynefazodone, metachlorophenylpiperazine (mCPP) and a triazoledione metabolite, all of which are pharmacologically active, is mediated by cytochrome P450 3A1 and 3A4. Nefazodone is a weak inhibitor of CYP2D6, the enzyme responsible for the metabolism of mCPP. In clinical studies, plasma concentrations ranged from 0.2-2.3 mg/L in subjects given 200 mg/b.i.d. The elimination half-life of hydroxynefazodone is much shorter than that for the parent drug ($t_{1/2}$= 3 h versus 18 h for nefazodone in normal metabolizers), while that of the triazoledione metabolite is much longer.

Nefazodone was identified by liquid chromatography-mass spectrometry (LC-MS) carried out at pH 10.0 using an ammonia/methanol/THF mobile phase (32.5:67.0:0.5, v/v) with a Zorbax Extend-C_{18} column (2.1 x 150 mm) following an n-butyl chloride extraction. Mass spectral detection was carried out using atmospheric pressure electrospray ionization (APESI) in positive mode. Ions with m/z 470, 471, and 472 were monitored for nefazodone and m/z 372, 373, and 374 were monitored for trazodone (IS). The limit of quantitation was 0.05 mg/L. In each of six postmortem cases where nefazodone was detected in blood, its specific pattern of distribution is reported. Concentrations ranged from <0.05-40 mg/L in all tissues, with individual tissue concentration ranges as follows: femoral blood: 0.13-4.7 mg/L, frontal cortex: <0.05-34 mg/L, bile: <0.05-22 mg/L, vitreous fluid: <0.05-0.99 mg/L, urine: <0.05-4.9 mg/L, and liver: 2.4-40 mg/L. With the exception of one case nefazodone was detected in the presence of other drugs, including other antidepressants, antipsychotics benzodiazepines, and opiates. As many antidepressants and some opiates are also substrates and/or inhibitors for CYP 2D6 or 3A4, the potential for competitive inhibition of metabolism exists. Nefazodone also has the potential to interact with other serotonergic drugs, and may contribute to the onset of serotonin syndrome. The specific mechanisms of interaction, as reported in the literature, are reviewed and evaluated in the investigation of six fatalities occurring over a two-year period. Three cases were classed as overdoses of one or more other drugs present, and in one case the pathologist cited serotonin syndrome as the cause of death. Postmortem redistribution may have occurred in two cases in which femoral blood concentrations exceeded the therapeutic range for nefazodone. There were no deaths ascribed solely to nefazodone intoxication.

Key words: Nefazodone, LC-MS, Postmortem Toxicology

12

Death due to Seizure or a New Antiepileptic Medication, Oxcarbazepine (Trileptyl®)?

Jeri D. Ropero-Miller*[1], Ruth E. Winecker[1], and Caroline L. Oldenburg[1], and David C. Winston[2]. [1]Office of the Chief Medical Examiner, Chapel Hill, NC 27599 U.S.A.; [2]Wake Forest University Baptist Medical Center, Winston Salem, NC 27157 U.S.A.*

Oxcarbazepine (Trileptyl®) is an anticonvulsant or antiepileptic (AED) drug utilized in the treatment of partial seizures. Approved by the Food and Drug Administration in 2000, oxcarbazepine at therapeutic doses of 600-1200 mg/d is effective without causing adverse effects associated with other antiseizure medications.

Limited information has been published regarding oxcarbazepine toxicity. Presently, there are no reported deaths in which the causative agent(s) includes oxcarbazepine and only recently have therapeutic postmortem fluid and tissue concentrations been reported. However, supratherapeutic postmortem concentrations have yet to be reported.

This report involves a 42 year-old woman who was found dead; lying supine and head-first on the staircase of her residence with her right leg caught in the banister. The decedent had a medical history of severe migraine headaches. Scene investigation revealed two empty syringes of Imitrex® and a note suggesting suicidal intentions. At autopsy significant findings included patchy bronchopneumonia, stress ulcers of the stomach, minor contusions / abrasions of the extremities, and surgical removal of the gall bladder. In addition, no obvious granular material was noted in the gastric content. Heart blood, vitreous humor, liver, urine and gastric contents were collected for toxicological analyses based on the death scene investigation and autopsy findings.

Toxicological analyses were negative for ethanol, carbon monoxide and common drugs of abuse. Screening and quantification of oxcarbazepine and its metabolite were performed by GC/FID and GC/MS following a solid-support, liquid-liquid extraction. The following drugs and their respective concentrations were detected in heart blood: Oxcarbazepine at 2.5 mg/L, 10-hydroxycarbazepine at 92 mg/L, and diphenhydramine at 0.97 mg/L. All remaining fluids and tissues analyzed for oxcarbazepine and its active metabolite, 10-hydroxycarbazepine had concentrations as follows: Oxcarbazepine- 730 mg/kg (liver), trace (vitreous), 7.6 mg/L (urine), and greater than 500 mg total (gastric) and 10-hydroxycarbazepine- 218 mg/kg (liver), 42 mg/L (vitreous), 400 mg/L (urine), and 3.8 mg total (gastric). These postmortem concentrations indicated that an acute dose of oxcarbazepine had been ingested prior to death. Were these toxicological findings consistent with a lethal ingestion of oxcarbazepine? Did the decedent fall down the stairs as a result of seizure activity or adverse effects of the drug? This interactive presentation asks the audience to share their conclusions before the cause and manner of death are revealed.

Key words: Oxcarbazepine, Antiseizure, Postmortem toxicology

13

Reported Death by Ingestion of 2,4-Dinitrophenol

Estuardo J. Miranda* *and Barry K. Logan. Washington State Patrol Toxicology laboratory, 2203 Airport Way S., Suite 100, Seattle, WA 98134-2027*

A 17-year-old female was admitted to a hospital presenting with nausea, diaphoresis and fever. The young woman was treated but subsequently died six hours after admission. An autopsy by the medical examiners office revealed no obvious signs for cause of death and the young woman's death was attributed to either septic shock or drugs. The medical examiner's office submitted a blood sample and two yellow powder capsules to the Washington state toxicology laboratory for complete drug analysis. The two capsules submitted were given to the medical examiner by the parents of the woman who had been taking them, without their knowledge, to loose weight.

Analysis of the blood demonstrated the absence of volatiles and basic drugs. Acidic and neutral drug analysis by gas chromatography-mass spectrometry (GC/MS) showed a significant peak with retention time of ~ 15 min and ion masses of 184, 154, 107, 91, 79, 63 and 53. This peak was identified as 2,4-dinitrophenol. Afterward, an aliquot of the yellow powder in the capsules dissolved in methanol was analyzed by GC/MS and shown to be 2,4-dinitrophenol. Furthermore, the acid/neutral extraction analysis of the powder also showed the presence of 2,4-dinitrophenol.

Dinitrophenols are used primarily as herbicides, fungicides, insecticides and wood preserver. This group of compounds has a common toxic mechanism: oxidation at the mitochondrial level is uncoupled from phosphorylation; metabolic rate is increased secondary to the deficit of adenosine triphosphate. Clinically there is a marked elevation of temperature, profuse diaphoresis and tachypnea. Earlier reports concerning dinitrophenol poisoning are of chronic toxicity because this agent was used for its metabolic effect in efforts to reduce weight. The signs and symptoms, with which the woman presented at the hospital, are consistent with acute dinitrophenol poisoning. Thus far, cases reported with acute dinitrophenol poisoning have not been able to quantify the levels present in either blood or serum.

Here we report the levels of 2,4-dinitrophenol present it the blood and serum in the woman presenting with symptoms of acute dinitrophenol poisoning. The blood and serum taken at the same time at the hospital were found to contain 36.14 mg/L and 29.68 mg/L of 2,4-dinitrophenol, respectively. According to the United Environmental Protection Agency 2,4-dinitrophenol is considered to be highly toxic to humans with a lethal dose of 14 to 43 mg/kg, which is consistent with our findings in this report. Following our finding the young woman's death was attributed to poisoning by 2,4-dinitrophenol.

Key words: 2,4-Dinitrophenol, Dinitrophenols, Poisoning

14

Fatal Overdose with Molindone and Lithium

*Dwight Flammia, Erik Christensen, Henry Bateman, and **Joseph J. Saady***. Virginia Division of Forensic Science, Richmond, VA*

Molindone is a dihydroindolone compound prescribed as the hydrochloride salt with the chemical name 3-ethyl-6, 7-dihydro-2-methyl-5-(morpholinomethyl) indol-4 (5H)-one hydro-chloride. This indolone drug has antipsychotic (frequently called neuroleptic) activity and is dissimilar in structure to other antipsychotic drugs (i.e. phenothiazines, butyro-phenones, dibenzapines, thioxanthenes). The antipsychotic drugs are used for the manage-ment of psychotic disorders, and particularly for the treatment of schizophrenia. Molindone hydrochloride (Moban®) is prescribed in tablet doses of 5, 10, 25, 50 and 100 mg as well as a concentrated solution in 4 ounce bottles (20 mg/mL). The starting dosage of molindone is between 50-75 mg/day and may be increased to 100 mg/day.

Molindone reaches peak concentrations in plasma at 30 to 60 minutes, and has a half-life of approximately 2 hours. Because of the short half-life it is typically administered two or more times per day. The clinical duration of action is at least 24 hours, which suggests that the antipsychotic activity may be mediated by active metabolite(s). Even at steady state doses of 100-150 mg per day, blood concentrations are negligible 12 hours after the last dose. The average blood level in psychiatric patients taking 100 mg/day is 0.044 mg/L 6 hours after the last dose. This case involves a 43 year old female with a long history of bipolar disorder, who was found collapsed in her residence. She had a history of prior hospitalizations for mental illness. Multiple prescriptions were found at the scene including empty prescription bottles of molindone, lithium, temazepam and thiothixene.

We report tissue concentrations of molindone in blood, liver, bile, gastric and urine as follows: blood 6 mg/L; liver 26 mg/Kg; bile 23.1 mg/L; gastric 1200 mg/L; urine 37.3 mg/L. Molindone quantitation was determined by buffering 2 mL of blood, bile, urine, gastric fluid or 2 grams of homogenized liver to a pH of 9.0 followed by liquid-liquid extraction using a mixture of toluene/hexane/isoamyl alcohol (v/v 78/20/2). Quantitation of molindone was performed using gas chromatography with nitrogen detection (GC-NPD). The range of linearity was 0.2-3.0 mg/L and molindone was provided by Endo Pharmaceuticals. Gas chromatography mass spectrometry (GC/MS) in scan mode was used for confirmation (m/z 100, 176, 276). The base screen also revealed benztropine present at less than 0.1 mg/L. Temazepam and thiothixene were not detected. Vitreous lithium was determined by flame atomic absorption spectrometry and found to be 5.9 mmol/L. The cause of death was acute molindone and lithium poisoning.

Key words: Molindone, Fatality, Lithium

15

Chemical Restraint of an Infant Using Diphenhydramine Associated With a Fatality

Andrew P. Mason*[1], Ruth Winecker[2], and Jeri Ropero-Miller[2]. [1]ToxicoLogics, Ltd., Boone, NC, U.S.A.; [2]Office of the Chief Medical Examiner, Chapel Hill, NC, U.S.A.*

Objective. To use a case report to describe the use of diphenhydramine (DPH) by a caregiver as a sedative-hypnotic in the chemical restraint of an infant in a day care setting, and to describe difficulties associated with interpretation of postmortem DPH concentrations in infants.

Results. "KS" was a 5-month-old female infant left in the care of "JB", the 62 year-old female proprietor of an unlicensed home day care facility. According to her parents, KS was not a "self-feeder", and was incapable of holding and manipulating a bottle. Nevertheless, she was placed in a "bouncy-seat" on her back, in a nearly recumbent position with a "propped" bottle, and was left in that position unattended, apparently so that she could feed without assistance from a caregiver. Upon the return of JB, KS was found to have vomited and expired. "Formula" was present on her face, and in her mouth and nose. Attempted resuscitation forced "formula" from the child's mouth and nose, but produced no vital response. There was a significant delay in summoning emergency medical support. Investigators found bottles of DPH syrup ("Equate Allergy Elixir, Alcohol Free") in the home, along with medicine cups and medicine droppers, one of which contained DPH residue. Parents of other children reported that their children were groggy, sleepy, lethargic, tired, or difficult to wake up after care at the facility.

Autopsy findings were insignificant except for evidence of foreign material in the lungs. Toxicology tests revealed DPH concentrations of 0.14 mg/L in aortic blood, 0.12 mg/L in blood from the vena cava, and 0.89 mg/kg in the liver. No alcohol or other organic bases were detected in aortic blood. According to her parents, they had never given DPH to KS, and had not given anyone permission to do so.

The blood concentrations detected in KS are consistent with DPH concentrations detected in peripheral plasma specimens associated with the production of sedation and hypnosis in adults in clinical studies [Clin Pharm Ther 23/4: 375-82, (1978)]. However, the relationships between these data (peripheral plasma concentrations in adults and central whole blood from an infant) could not be determined. Other difficulties associated with interpretation included an inadequate database of DPH concentrations in infants in "therapeutic" and "toxic" (as opposed to fatal) circumstances, inadequate information regarding the pharmacokinetics of DPH in infants, and inadequate knowledge of the influence of post-mortem distribution phenomenon in infants. Difficulties determining the relative timing of the apparent aspiration (antemortem, perimortem-agonal, or postmortem as an artifact from attempted resuscitation by the untrained caregiver) complicated the assignment of cause and manner of death. Because of these many difficulties, the administration of DPH as a causal or contributory influence on the production of the fatality could not be supported to a reasonable degree of scientific certainty. The care-giver later plead guilty to three charges, child abuse and neglect, assault on a child under 12 years old, and operating an unlicensed day-care center, all misdemeanor charges.

Conclusions. DPH was used for the purpose of chemical restraint in an infant. However, the determination of DPH administration as a causal or contributory factor in the fatality proved to be problematic due to a lack of data for comparative interpretation.

Key words: Diphenhydramine, Infant, Chemical restraint

16

Tissue Distribution of Xylazine in a Suicide by Hanging

Karla A. Moore*, *Mary G. Ripple, Saffia Sakinedzad, Barry Levine, and David R. Fowler. Office of the Chief Medical Examiner, State of Maryland, 111 Penn St., Baltimore, MD 21201*

Xylazine (Rompun®, Sedazine®, AnaSed®) is currently the most commonly used sedative-analgesic in veterinary medicine. There are nine published cases of xylazine's involvement in human drug-related deaths and impairment. However, blood concentrations have been reported in only four of these cases. Three of these nine cases were fatalities involving xylazine, two of which involved xylazine alone but did not report blood concentrations due to extensive decomposition of the bodies. This report documents a case where xylazine alone was identified in a suicide by hanging.

The deceased was a 42-year old, Caucasian male, a practicing veterinarian with a history of depression. He had left a note for his wife stating he "...could be found hanging from a tree at the gravel quarry...". A used 3 cc syringe, clamps and a tourniquet were found on the ground below the body. At autopsy, the body presented with a rope around the neck. There was an underlying ligature furrow mark on the anterior aspect of the neck, extending upwards behind the ears to the occipital area. Rare petechiae were identified on the conjunctive bilaterally. There were no other significant autopsy findings.

Xylazine was detected using an alkaline drug extraction followed by analysis on a Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen-phosphorus detector (NPD). The presence of xylazine was confirmed in the urine by full scan electron ionization gas chromatography/mass spectrometry. The mass spectrum of xylazine has a base peak of m/z=205, with other prominent ions at m/z=220, 177 and 145. The following xylazine concentrations were found: heart blood 2.3 mg/L; peripheral (subclavian) blood 2.9 mg/L; bile 6.3 mg/L; urine 0.01 mg/L; liver 6.1 mg/kg and kidney 7.8 mg/kg.

We believe this case is unique as we report blood concentrations in a case where no other drugs were detected and where xylazine was not the cause of death. While the exact amount administered was unknown, the maximum amount that could have been administered is 300 mg since a 3-cc syringe was found and the highest concentration available is 100 mg/mL. Assuming the maximum dose possible, this equates to a dose of ~3.5 mg/kg in this 89 kg individual. This is well within the range of non-fatal doses reported elsewhere. The detection of a significant amount of drug in the tissues, including the bile and a small amount in the urine, may indicate a period of survival before his death, ultimately by strangulation.

Key words: Xylazine, Suicide, Tissue distribution

17

Fatalities Involving Hydroxychloroquine: Chronic Accumulation?

Peter Singer* *and Graham Jones. Medical Examiner's Office, Edmonton, Alberta, Canada*

Hydroxychloroquine is used to treat malaria, lupus and severe arthritis. The drug is not commonly found in Medical Examiner's cases and there are few reports in the literature where hydroxychloroquine is associated with fatality. Consequently, when such cases do occur, it is difficult to interpret the findings. Four fatal cases are reported with the following postmortem hydroxychloroquine concentrations.

Case 1 A 54 year old woman with lupus was found to have 43 mg/L in femoral blood and 500 mg/kg in liver; no other drugs were detected. An overdose was not suspected.

Case 2 A 33 year old woman with a history of arthritis and drug abuse died shortly after admission to hospital due to severe epigastric pain. She was found to have 24.7 mg/L in femoral blood and 183 mg/kg in the liver, plus small amounts of methylphenidate and pentazocine. However, there was no evidence to suggest an acute overdose.

Case 3 This 57 year old woman with a history of heart disease and arthritis collapsed at home and died shortly afterwards in hospital. She was found to have hydroxychloroquine concentrations of 6.1 mg/L and 96 mg/kg in jugular blood and liver respectively. Also present were significant amounts of ethanol, oxycodone and diphenhydramine. The death was attributed to a combined drug intoxication. There was no evidence to support a suicidal overdose.

Case 4 This 59 year old woman was found dead at home. Death was attributed to a combined codeine/morphine intoxication. The finding of 11.2 mg/L hydroxy-chloroquine in cardiac blood was judged, at the time, to be incidental.

Hydroxychloroquine was detected by gas chromatography/mass spectrometry (GC/MS) and quantified by liquid chromatography/MS (LC/MS). Blood or tissue homogenate to which chloroquine has been added as internal standard was buffered to pH 12 and extracted with chlorobutane. The analytes were back-extracted into 0.1N H_2SO_4, the acid layer basified an the re-extracted into chlorobutane. The organic extract was evaporated and the residue reconstituted in mobile phase. A 30 cm Zorbax XDB C_8 LC column was used and the mobile phase was acetonitrile / 50 mM ammonium acetate. Ions 320, 247, (and chlorine isotope ions at 322, 249) were monitored for hydroxychloroquine, and 336, 247, (and chlorine isotope ions at 338, 249) for chloroquine, in positive ion electrospray mode. Typically, a blood-based 6-point calibration over the range 0.5 to 20 mg/l was linear (r^2 of 0.999) with a x-intercept of -0.02.

Suicidal hydroxychloroquine overdose was judged not to be likely in our cases, despite the fact that the concentrations are comparable to the few literature reports attributed to deliberate overdose. Chronic accumulation of hydroxychloroquine due to impaired excretion, inhibition of metabolizing enzymes, the extremely long half-life (and for blood, postmortem redistribution) are considered likely factors in explaining the unexpectedly high hydroxychloroquine concentrations.

Key words: Hydroxychloroquine, Accumulation, Fatality

18

Fatal Acute Topiramate Toxicity

Loralie J Langman[1], *Henry A Kaliciak[1]*, *Sharon A Boone[2]*. *[1]Provincial Toxicology Centre, Riverview Hospital, Port Coquitlam, BC Canada, [2]Forensic Pathology Unit, Royal Columbian Hospital, New Westminster, BC Canada*

Topiramate is an antiepileptic agent approved for use as adjunctive therapy in the treatment of adults with partial-onset generalized seizures that are not controlled by monotherapy. We present what appears to be the first documented case of a fatality due to a massive overdose of topiramate.

A 44 year old Caucasian female was found dead in bed. A full autopsy was performed approximately 48 hours after death. Autopsy findings included pulmonary edema, mucous plugging of small airways, and cerebral edema. Specimens were collected for Toxicological analysis.

All specimens were initially subjected to a thorough qualitative analysis. Screening was performed for basic, acidic, neutral drugs, and volatiles by standard chromatographic methods. Qualitative analysis identified ethanol, phenobarbital, methotrimeprazine, and topiramate. The ethanol concentration was 0.08 % (17 mmol/L) in blood, 0.18 % (39 mmol/L) in urine and 0.13 % (28 mmol/L) in vitreous fluid. The phenobarbital concentration was 16 mg/L (68 mmol/L) and was within its therapeutic limit (10 - 40 mg/L). The metho-trimeprazine concentration was 0.78 mg/L (2.4 mmol/L) and was above its therapeutic limit (0.051 - 0.141 mg/L). Neither level was sufficient to attribute as the cause of death.

Topiramate was assayed in biological specimens as follows: briefly, to 1 mL of specimen 10 uL of droperidol solution (internal standard, final concentration 10 mg/L) was added, and extracted into 3 mL of acetonitrile:water (9:1) solution. The supernatant (1 µL) was injected directly into an Agilent model 1090 liquid chromatograph coupled to an Agilent Model 1100 mass Spectrometer using a Zorbax C8 4.6 x 30 mm 3.5 µm column (Agilent). The mobile phase consisted of a mixture of solvent A [1.0 mmol/L ammonium acetate aqueous buffer with 0.1% trifluoroacetic anhydride] and solvent B [1.0 mmol/L ammonium acetate in acetonitrile with 0.1% trifluoroacetic anhydride]. Separation was achieved using a binary solvent gradient. The initial conditions are 10% Solvent B, after 1 minute the solvent mixture was 50% Solvent B and returning to 10% after 3 minutes. The solvent ratio was held isocratically at 10% Solvent B until the end of run at 6 min. The flow rate was constant at 0.50 mL/min. Detection was by mass spectrometry in electrospray mode using single ion monitoring. The ions used were as follows: for topiramate – 340 m/z, and for droperidol – 380 m/z. The concentration was measured by comparison of peak height ratio of topiramate to that of droperidol against a standard curve. Linearity was observed up to 100 mg/L. Samples with concentrations exceeding the linearity were diluted.

Elevated concentrations of topiramate were found. Analysis of available biological fluids and tissues was carried out with the following results: blood (central) 170 mg/L, liver 140 mg/kg, stomach contents greater than 2000 mg/L, and vitreous fluid 65 mg/L. Serum levels in living subjects have been reported to range form 5.9-35 mg/L. The specimen concentrations in this case were nearly 5 fold the therapeutic range, assuming the red blood cell serum distribution ratio is 1:1. The cause of death was ascribed to solely topiramate overdose.

Key words: Topiramate, Fatality, Overdose

19

Fatal Inhalation of Glade® Air Freshener in a 12-Year Old Girl in Arizona

Diane J. Mertens Maxham*, Charles E. Spies and Norman A. Wade. Office of the Medical Examiner, Maricopa County, Phoenix, AZ, U.S.A.*

A 12-year old American Indian girl was reportedly "huffing" Glade® Air Freshener (S.C. Johnson & Son, Inc.) with friends, when she hallucinated, (yelling "Get them off of me!"), collapsed, and subsequently died. Upon toxicological examination, three distinct peaks were noted in the volatile headspace analysis of her femoral blood sample. These same peaks were also found in headspace analysis of her brain tissue. Further examination revealed these to be the same peaks (propane, isobutane and butane) found in the Glade® "Cinnamon Sticks" air freshener that was brought in as evidence with her body.

Inhalation of volatile substances is a well-documented problem, especially in the American Indian youth population of the southwest USA. Our office has reported 15 cases since 1996 in which inhalant abuse has contributed to the cause of death. Of these, 10 cases involved persons aged 18 or under. Often, the inhaled volatile compounds are the only toxicological findings present. Effects of inhalation abuse include dizziness, confusion, hallucinations, and loss of coordination. Death occurs as a result of cardiac arrhythmia (ventricular fibrillation) and hypoxia.

Routine headspace volatile analysis (100 μL of sample added to 1 mL internal standard, heated for 10 min. at 50°) usually indicates the presence of these hydrocarbons as early eluting peaks. Samples were analyzed using a Hewlett Packard 7694 Headspace Autosampler on an HP 5890 GC with a 20 meter 5% Carbowax column, and detected by FID at isothermal (80°) oven conditions. Confirmation of the peaks found in Glade® (as noted on the MSDS sheet) was accomplished using Supelco SPME (Solid Phase Micro Extraction) by HP 5970 MSD/5890 GC.

In conclusion, the volatile substances in this case were observed in the femoral blood and brain tissue, but were not found in the vitreous humor or tracheal air. Therefore, it is important for the Medical Examiner to promptly indicate the potential inhalant abuse cases to the lab, and collect appropriate samples (brain, lung, liver, and kidney tissue) for the laboratory to analyze.

Key words: Inhalant abuse, Headspace, Volatiles

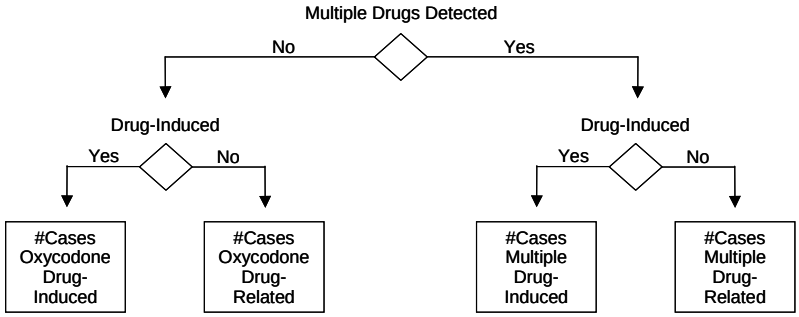
20

Standardizing Terminology for Reporting Cause of Death in Cases Involving Opioid Abuse

Yale H. Caplan*[1], *John M. Pinney*[2], *Edward J. Cone*[3]. [1]*National Scientific Services, Baltimore, MD;* [2]*Pinney Associates, Bethesda, MD;* [3]*ConeChem Research, Severna Park, MD*

Given the public health impact of drug abuse, there is a clear need for the use of standardized terminology in reporting mortality data on drug abuse-related deaths. Reporting mortality data involving opioid-induced and opioid-related deaths is particularly problematic. Fatalities attributed to overdose are likely to have opioid concentrations no higher than those found in regular opioid abusers, or those who die from other causes. Concomitant self-administration of other depressant drugs is common practice among opioid abusers. This practice substantially increases the likelihood of a fatal outcome, due to the potentiation of respiratory depressant effects of opioids. Limiting cause of death to a single opioid ignores the "likely causal contribution of other drugs to the mechanism of death" (Darke and Zador, 1996). The most successful effort in establishing standardized terminology for reporting mortality data has been made by the U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration (SAMHSA) through its Drug Abuse Warning Network (DAWN). We propose a model that utilizes standardized terminology for reporting cause of death (COD) in cases involving opioids. To be reportable the case must meet the following criteria: a) drug-induced (one or more drugs directly caused the death) or drug-related (drug abuse was a contributing factor, but was not the sole cause of death, e.g., drug abuse in combination with a physical event such as an automobile accident or a medical disorder such as hepatitis that was caused by drug abuse); b) decedent used drugs to commit suicide or achieve psychic effect. Importantly, attributing COD to any particular drug in polydrug-abuse cases (cases that involve abuse of opioids in combination with other drugs) is difficult and cases should be reported as a "multiple-drug" death. The following model, which utilizes oxycodone as an example, is proposed for reporting COD statistics for deaths involving opioid abuse:

Model for Reporting COD in Cases Involving Oxycodone Abuse



Adoption of standardized terminology that is consistent with other government drug abuse reports would more accurately reflect COD and contribute to more accurate interpretation and reporting of mortality data.

Key words: Mortality data, Opioids, Standardized terminology

21

Forensic Entomotoxicology: A Study of the Effects of Barbiturates and Amphetamines on the larvae of *Phaenicia sericata*

Michelle R. Peace*[1], *Alphonse Poklis*[1], *Jason H. Byrd*[2]. [1]*Department of Pathology, Medical College of Virginia Campus at the Virginia Commonwealth University, Richmond, VA 23298,* [2]*Department of Biology, Virginia Commonwealth University, Richmond, VA 23284*

Post-mortem intervals (PMI) can be estimated accurately, sometimes within a few hours of the actual time of death, using insect life cycles and successions as measuring tools. If the deceased individual consumed any drugs prior to death, then those drugs could affect the behavior and physiology of insect larvae. The purpose of this research was to determine the effect of barbital, phenobarbital, pentobarbital, thiopental, ephedrine, methamphetamine, and fenfluramine on the duration of the larval stage of the *Phaenicia sericata* life cycle and the subsequent mass and length of their mature larvae. *P. sericata* were wild-trapped and maintained in a laboratory colony at a controlled temperature and light cycle. A blank control solution, 0.5 LD_{50}, 1.0 LD_{50}, and 2.0 LD_{50} of each drug (for rabbits) were homogenized in ground pork loin. Fly eggs were removed from the adult cages, and approximately 80 were placed onto each sample of ground pork and reared at 22^{o}C. Once the mature maggots began to migrate, they were harvested, cleaned, and frozen.

The duration of the larval stage was measured from the day that the larvae hatched to the day they began to migrate. The duration of the larval stage in the control sample was 5 days. Almost every concentration of each drug in the barbiturate class shortened the larval stage to 4 days. Barbital and phenobarbital at 2.0 LD_{50} caused severe stunting of larval growth, high mortality, and no migration of larvae. Every concentration of ephedrine and fenfluramine lengthened the larval stage to 6 days. Methamphetamine at 0.5 and 1.0 the LD_{50} lengthened the larval stage to 6 days while 2.0 LD_{50} lengthened the larval stage to 7 days. Additionally, length and weight measurements of larvae feeding on the drug laden pork loin showed that the drugs significantly ($n=25$, $p<0.05$) affected the lengths and weights of the larvae when compared to the controls. Barbital caused larval weights to decrease while lengths remained consistent with the control, and phenobarbital, methamphetamine and ephedrine caused larval lengths to increase while the weights remained relatively consistent. These preliminary results indicated that PMI estimation based upon insect life cycles could be significantly affected if the body on which the larvae fed had consumed any drugs.

Key words: Entomotoxicology, Barbiturates, Amphetamines

22

Analysis of 6-Monoacetyl Morphine, Morphine, Codeine and Dihydrocodeine in Oral Fluid Collected from a Population of Drug Users

M.D. Osselton and S. Robinson. UK Forensic Science Service, Chepstow Laboratory, Usk Road, Chepstow, South Wales, UK.*

As part of an evaluation of an on-site oral fluid (OF) test for opiates and cocaine, specimens that provided a positive opiate screening result were analysed by GC/MS for the presence of 6-monoacetyl morphine (6-MAM), morphine, codeine and dihydrocodeine. On-site screening was undertaken using the Cozart RapiScan with a cut-off for screening of 10 ng/ml (equivalent to 30ng/ml in undiluted OF). Specimens indicated as opiate positive were extracted by automated solid phase extraction (SPE) using IST HCX 130mg/3ml columns on a Hamilton 4000 SPE robotic workstation, with d3-morphine, d3-codeine and d3-6-MAM as internal standards. Extracts were derivatised using BSTFA and analysed by GC/MS in SIM mode using an Agilent 5973 MSD / 6890 GC, fitted with a HP-5ms cross-linked fused capillary column (30m x 0.25mm id with 0.25 μm film thickness) and splitless mode injection. Opiate positive OF specimens (331) were analysed for codeine, dihydrocodeine, morphine and 6-monoacetyl morphine.

Opiate concentrations ranging between 0.03 mg/L - 2.9 mg/L for 6-MAM, 0.02 mg/L - 2.98 mg/L for morphine, 0.027 mg/L - 2.2 mg/L for codeine and 0.03 mg/L - 2.6 mg/L for dihydrocodeine were detected. This study shows that it is possible for the concentrations of these substances to reach exceptionally high concentrations in the oral fluid of regular drug users. The high concentrations of 6-MAM, morphine and codeine in oral fluid may indicate that drug depots are being formed in mouth tissues, possibly as a result of smoking heroin. However, the situation may not be quite so simple since dihydrocodeine was detected over a similar concentration range yet is not smoked. Dihydrocodeine is available as a prescription only medicine in the UK in tablet, linctus and injectable formulations. Control experiments performed using over the counter preparations containing codeine and opium tincture showed that deliberate oral contamination with these drugs dissipates rapidly.

Key words: Oral fluid, Opiates, Drug users

23

An Assessment of the Potential for Contamination of the PharmChek™ Sweat Patch

Dennis J. Crouch*, *Jakub Baudys and Matthew H. Slawson. Center for Human Toxicology, University of Utah, Salt Lake City, UT*

Several studies have shown that the PharmChek™ sweat patch is particularly useful for drug testing in criminal justice settings. However, there have been questions about the effectiveness of the skin cleansing procedures used prior to application of the patch and the susceptibility of the patch to contamination from vaporized drugs. To address the cleansing question, subjects were recruited and placed in 2 study groups. **Study Group 1** subjects had a water solution containing cocaine (Coc) and methamphetamine (Meth) (1000 ng) sprayed onto their upper arm. After the solution was dried, the area was cleansed twice with separate alcohol wipes and a sweat patch was applied. **Study Group 2** subjects were treated the same except the area was cleansed once with the 5% soap solution, once with tap water, and finally once with an alcohol wipe and the patch applied. Each group had a similar "control" area prepared on their opposite arm. Subjects returned 7 days later for harvesting of the sweat patches. In subsequent visits, 500 ng and 100 ng of the drugs were applied. There was a relationship between dose and residual drug detected on the patch. The number of drug-positive patches followed the trend 1,000 ng dose > 500 ng dose > 100 ng dose. The concentration(s) of the drugs detected on the patches also followed this trend. Cleansing with soap + isopropanol was more effective at removing the residual drug from the subjects skin than 2X isopropanol wipes. Fewer patches tested positive and drug concentrations/patch were lower following the soap/isopropanol procedure. Drug and metabolite were not detected in the same patch following the 1,000 ng, 500 ng or 100 ng treatment regardless of wash procedure. The DHHS suggested administrative reporting cutoff concentration of 25 ng/patch, was effective in reducing the possibility of residual drug on the skin resulting in a positive sweat patch result. No benzoylecgonine (BZE) or amphetamine (Amp) concentrations exceeded this cutoff. Also, if a policy is established that both drug and metabolite must be detected in the patch, no patches (treated or control) would have been reported as positive in this study.

To address the question of vaporized drug contaminating the patch, intact patches were exposed to vapors from 10 and 20 mg of Coc and 5 and 10 mg of Meth (free base) in a closed container. The absorptive pad was treated as follows: dry; or moistened with 100 mcL artificial sweat, drug-free sweat or water. The external surface of the patch was dry or moistened with 100 mcL of artificial sweat, pH 4.0 buffer, or pH 9 buffer. The polyurethane membrane of the sweat patch served as an effective barrier to vaporized Coc and Meth. At external membrane pHs from 4 to 9, Coc and Meth did not readily pass through the membrane when moderate doses of drug were vaporized (regardless of the pad condition). It was only with extreme experimental conditions (not indicative of the most extreme wear conditions) that Coc and/or Meth were detected in the pad. When the membrane barrier was in place, Coc concentrations only exceeded the proposed DHHS cutoff in two pads. Meth concentrations consistently exceeded the 25 ng/patch cutoff following the high dose exposure. However, employing the administrative criterion of detecting both parent drug and metabolite to report a positive, no patches would have been reported positive from vapor phase exposure.

Key words: Sweat patch, Cleansing, Drug vapor

24

Oral Fluid Testing for Drugs of Abuse: Positive Prevalence Rates and Suggested Cutoff Concentrations

***Edward J. Cone*[1]**, *Lance Presley*[2], *Michael Lehrer*[2], *William Seiter*[2], *Melissa Smith*[2], *Keith W. Kardos*[3], *Dean Fritch*[3], *Sal Salamone*[3], *R. Sam Niedbala*[3]. [1]*ConeChem Research, LLC, Severna Park, MD;* [2]*LabOne, Inc., Lenexa, KS;* [3]*OraSure Technologies, Bethlehem, PA*

Draft guidelines for the use of oral fluid for workplace drug testing are under development by SAMHSA in cooperation with industry and researchers. Comparison studies of the effectiveness of oral fluid testing versus urine testing are needed to establish scientifically reliable cutoff concentrations for oral fluid testing. We present the results of the first large scale database on oral fluid testing in private industry. A total of 77,218 oral fluid specimens were tested over the period of January through October, 2001 at Lab*One*, Lenexa, KS. Specimens were screened by Intercept immunoassay at manufacturer's recommended cutoff concentrations for the five SAMHSA drug categories (marijuana, cocaine, opiates, phencyclidine and amphetamines). Presumptive positive specimens were confirmed by GC-MS-MS. A total of 3908 positive tests were reported over the ten-month period representing a positivity rate of 5.06%. Of the five drug categories, marijuana and cocaine accounted for 85.75% of the total positives. The pattern and frequency of drug positives showed remarkable similarity to urine drug prevalence rates reported for the general workforce according to the Quest Diagnostics' Drug Testing Index over the same general period suggesting that oral fluid testing produces equivalent results to urine testing. The data on oral fluid testing also revealed a surprisingly high 66.7% prevalence of 6-acetylmorphine confirmations for morphine positives suggesting that oral fluid testing may be superior in some cases to urine testing. Comparison of oral fluid drug concentrations to SAMHSA recommended cutoff concentrations in Draft Guidelines indicated that adoption of the screening and confirmation cutoff concentrations of Draft Guidelines #3 would produce the most accurate reporting results with the exception of amphetamines. Consequently, it is suggested that the final Guidelines adopt the screening and cutoff concentrations listed in Draft Guidelines #3 with the exception of lowering the amphetamines cutoff concentrations (screening/confirmation) to 50/50 ng/mL for amphetamine and methamphetamine.

Key words: Oral fluid, Prevalence, Cutoff concentrations

25

Elimination of 7-Aminoclonazepam in Urine After a Single Dose of Klonopin™

Adam Negrusz*[1*], *Andrew M. Bowen*[1], *Christine M. Moore*[2], *Sheila M. Dowd*[1], *Mary Jane Strong*[1], *Philip G. Janicak*[1], [1]*University of Illinois at Chicago Medical Center, Chicago, IL 60612,* [2]*United States Drug Testing Laboratories, Inc., 1700 S. Mount Prospect Rd., Des Plaines, IL 60018

The objective of this paper was to determine how long after administration of benzodiazepine clonazepam (CLO), the drug and its major metabolite 7-aminoclonazepam (7ACLO) could be detected in urine collected from 10 healthy volunteers who received a single 3 mg dose of Klonopin™ (CLO). Such data would be of great importance to law enforcement agencies trying to determine the best time interval for hair collection from a victim of drug-facilitated sexual assault in order to reveal drug use. To achieve the above goal, highly sensitive NCI-GC-MS method for the simultaneous quantitation of CLO and its major metabolite 7ACLO in urine was developed and validated. Ten healthy volunteers (6 women and 4 men, 23-49 years old) participated in the study. The following urine samples were collected from each volunteer: one before CLO administration, 6 hours, and 1, 3, 5, 8, 10, 14, 21 and 28 days after. All urine samples (1 ml) were extracted following addition of the internal standard (D_5 diazepam) and enzymatic hydrolysis (∃-glucuronidase) using HCX (200 mg, 10 ml) solid phase extraction columns. Drugs were eluted from the column using methylene chloride:isopropanol:NH_4OH (78:20:2, v/v/v). Extracts were evaporated to dryness, and derivatized with HFBA (50 ul). HFBA was evaporated and ethyl acetate (25 ul) was added. A Hewlett Packard GC-MS system comprising a 6890 GC and a 5973 MSD (CI with methane) was operated in SIM mode with splitless injection. For CLO *m/z* 315 and 279, for 7ACLO *m/z* 461 and 463, and for D_5 diazepam *m/z* 289 ions were monitored. Standard curves for CLO (500-4000 pg/ml) and 7ACLO (50-2000 pg/ml) were prepared by spiking aliquots of negative urine and had correlation coefficients of 0.998 and 0.982, respectively. In addition, two levels of control urine samples were prepared for CLO and 7ACLO. All method validation parameters were within acceptable limits.

The urine from every subject was still positive for 7-ACLO fourteen days after adminis-tration of the drug. Eight of the ten volunteers had measurable amounts of the metabolite twenty-one days after administration. One volunteer was still positive twenty-eight days after administration. Six of the volunteers had urine concentrations that peaked at one day after administration. One volunteer had the highest concentration of 7-ACLO at three days, two volunteers at five days, and one at eight days. The range of concentrations detected was from 73.0 pg/ml to 183.2 ng/ml. CLO was not detected in any of the samples.

Key words: Drug-Facilitated sexual assault, Clonazepam, Solid Phase Extraction, NCI-GC-MS

26

Determination of Time of Last Exposure Following Controlled Smoking of Multiple Marijuana Cigarettes

Huestis MA*[1], *Zigbuo E*[1], *Heishman SJ*[1], *Preston KL*[1], *Moolchan EM*[1], *Nelson RA*[1], *Newton JF, Gorelick DA*[1]. *National Institute on Drug Abuse, 5500 Nathan Shock Drive, Baltimore, MD, 21224; *[2]Sanofi-Synthlabo Pharmaceuticals, Inc., Malvern, PA 19355*

Objectives: The objective of this work was an evaluation of two mathematical models for the prediction of elapsed time after marijuana smoking following controlled administration of multiple marijuana cigarettes. Model I is based on the plasma concentration of delta-9-tetrahydrocannabinol (THC) and Model II is based on the ratio of the plasma concentration of the inactive metabolite, 11-nor-9-carboxy-THC (THCCOOH) to THC. The models have been successfully employed in many forensic investigations around the world, primarily in driving under the influence cases. Plasma data from controlled administration of single smoked doses were used to develop the models; in the present study, we further challenged the models' ability to accurately predict time of use after one and two marijuana cigarettes.

Methods: Healthy male marijuana users (N=38) provided informed consent to participate in this NIDA IRB approved protocol. Subjects received oral SR141716, a CB1-cannabinoid receptor antagonist, and then smoked two 2.64% THC cigarettes two and six h later. Nine of 38 subjects did not smoke the second cigarette due to either medical disqualification or personal choice. Plasma was collected immediately after and up to 6 h after marijuana smoking. Paired plasma THC and THCCOOH concentrations (N=717) were determined by SPE followed by negative chemical ionization GC/MS with limits of quantitation (LOQ) of 0.5 ng/mL for THC and 2.5 ng/mL for THCCOOH. Actual times of last use were compared to the 95% CI predicted by the models. Accuracy was defined as 100 times the number of correct predictions divided by total predictions. The percentages of over and underestimations and average time over or underestimated were also calculated.

Results: Model I accurately predicted time of use in 94.3% of 717 cases. Of the 41 samples where the time of use was outside the predicted interval, Model I overestimated time of use in 38 cases with an average overestimate of 17 min (range 1 to 50) and underestimated on average by 5 min the time in 3 cases (range 1 to 11). Model II accurately predicted the elapsed time after marijuana smoking in 96.6% of 704 cases (THCCOOH was <LOQ in 13 specimens). Time of use was underestimated in 21 cases with an average error of 38 min (range 1 to 96) and overestimated in 3 cases, an average of 3 min (range 1 to 4). For the 290 specimens collected after the 2nd smoked cigarette, Model I accurately predicted time after smoking in 92.4% of the cases; in 22 cases the time of last use was overestimated with no underestimations. Model I predictions were 99.0% accurate after the 2nd cigarette with three overestimates and no underestimates.

Conclusion: These data extend the previous validation of Models I and II for predicting the time of marijuana smoking from plasma cannabinoid concentrations following multiple smoked doses.

Key words**:** Prediction models; Estimation of time of marijuana use; Plasma cannabinoids

27

Amphetamine Excretion Following Administration of Adderall

John T. Cody*[1], *Sandra Valtier[2] and Steve Nelson[3]. [1]Academy of Health Sciences, Ft. Sam Houston, TX, [2]Clinical Research Squadron, Wilford Hall Medical Center, Lackland AFB, TX, [3]Department of Pediatrics, Wilford Hall Medical Center, Lackland AFB, TX*

Amphetamine remains a widely abused drug throughout the world. It is also used therapeutically for weight loss, narcolepsy and attention deficit disorder with hyperactivity (ADHD). ADHD has grown dramatically recently both in terms of diagnosis and treatment. Increasingly, older individuals are diagnosed and treated for ADHD and treatment often continues into adulthood. Of the available treatments for ADHD, Adderall (Shire Richwood) is widely prescribed. Despite its wide use, there are no published data regarding the expected amphetamine excretion profile following its use. This is problematic since, in this case, medical review officers (MRO) and toxicologists are asked to assess results in terms of use pursuant to valid medical prescription without specific data on which to base a sound decision**.**

To address this lack of information in the scientific literature, a study to determine the concentration and enantiomer composition of amphetamine following administration of Adderall was undertaken. Adderall (20 mg) was administered to five healthy subjects with all subsequent ad lib urine samples (total urine void) collected for seven days. Adderall is a 3:1 mixture of d- and l-enantiomers of amphetamine salts. Because the enantiomers are metabolized at different rates, their proportion offers the opportunity to describe excretion versus time. Coupling this data with drug concentration makes it possible for forensic toxicologists and MROs to come to an informed decision about the involvement of this drug in a positive result.

Urine samples were analyzed for amphetamine using liquid-liquid extraction. Samples were derivatized with N-trifluoroacetyl-l-prolyl-chloride to determine the enantiomeric profile and heptafluorobutyric anhydride was used for quantitative analysis. GC/MS analysis was by electron ionization selected ion monitoring. Samples were also analyzed for creatinine and specific gravity as indicators of the degree of dilution and for pH due to its significant effect on the excretion of amphetamine.

Results of the 252 samples collected in this study revealed substantial amounts of amphetamine were excreted. Peak concentrations ranged from approximately 2,650 – 5,950 ng/mL. Samples containing $\geq$ 500 ng/mL of amphetamine (the administrative cutoff for a positive result) were seen up to 47:30 hours post dose. The number of positive samples ranged among individuals from 7 – 13. As anticipated, analysis showed the d-enantiomer to be in excess of the l-enantiomer with the proportion of l-enantiomer increasing over time. The drug concentration profiles were quite variable within and between subjects, however, taking into account the dilution and pH of the samples, much of the wide fluctuations were accounted for.

These results are the first to describe the excretion of amphetamine following administration of Adderall. The presence of the l-enantiomer separates this drug from other preparations of the drug that are composed of only the d-enantiomer (i.e. Dexedrine and much illicit amphetamine), thus readily differentiating them from Adderall use. Some illicit and medicinal amphetamine is, however, a mixture of amphetamine enantiomers. Using the combination of enantiomer composition and quantitative data will allow MROs and toxicologists to better assess the use of this drug from abuse of amphetamine.

Key words: Amphetamine, Adderall, Enantiomers

28

Metabolism of Buprenorphine at Therapeutic Concentrations in Human Liver Microsomes and cDNA-Expressed Human Liver Cytochrome P450s

David E. Moody*, Matthew H. Slawson, and Paul A. Bemis. Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112*

Buprenorphine is a partial mu agonist/kappa antagonist used to treat moderate to severe pain and is currently being investigated for use in replacement therapy for opioid dependence. A major oxidative metabolite of buprenorphine is norbuprenorphine. Previous studies using cDNA-expressed cytochrome P450s (P450s) and selective P450 inhibitors have indicated the involvement of P450 3A4 in the N-demethylation of buprenorphine to norbuprenorphine. Two limitations to these studies were addressed in the current study. First, the previous studies did not use buprenorphine concentrations less than 2.5 µM, while therapeutic concentrations approach 0.02 µM. Second, the involvement of P450 3A4 was not total, as 25% of the activity could not be inhibited with ketoconazole, a selective inhibitor of P450 3A4. In addition, earlier studies provided evidence for oxidative metabolites besides norbuprenorphine. By comparing buprenorphine utilization to nor-buprenorphine we hoped to qualitatively address this issue also.

We have now used our sensitive and selective LC-MS-MS method for buprenorphine and norbuprenorphine to initiate studies on the in vitro metabolism of buprenorphine at substrate concentrations that approach therapeutic concentrations. Human liver microsomes were incubated with concentrations of 5, 21 and 82 nM buprenorphine for 5, 10, 20 and 30 minutes. Linear rates of norbuprenorphine formation and buprenorphine utilization were observed out to 10 minutes. Norbuprenorphine formation was time and substrate concentration dependent. In all cases buprenorphine utilization exceeded maximum nor-buprenorphine formation by 3 times, strongly suggesting that norbuprenorphine was not the only product being formed.

Buprenorphine (21 nM) was then incubated with a number of cDNA-expressed P450s for 20 minutes. Norbuprenorphine formation (rates in parentheses) was detected with P450s 2C8 (10), 3A5 (10), 3A4 (7.5), 3A7 (3.5), 2C18 (1.2) and 2C19 (1.0). Buprenorphine utilization for 2C8 was similar to norbuprenorphine formation, but was approximately twice that of norbuprenorphine formation for P450s 3A5, 3A4, 3A7, 2C18 and 2C19. In addition, buprenorphine utilization was observed with P450s 2D6 and 2E1 that did not produce norbuprenorphine.

These studies show that P450 3A4 plays a major role in the metabolism of buprenorphine to norbuprenorphine and other metabolite(s) at therapeutic concentrations. They also show that P450 2C8, and other members of the 3a family, have similar activities, and that norbuprenorphine only accounts for 33 to 50% of buprenorphine oxidative metabolism. Future studies will attempt to identify the other metabolite(s) and assess the quantitative role of different P450s in their formation. (Supported by NIDA R01-DA-10100).

Key words: Buprenorphine, Metabolism, Cytochrome P450s

29

Recent Experiences with Incidences of Ethylene Glycol Poisoning in the State of Georgia

Christopher S. Tilson*, *and H. Horton McCurdy. Georgia Bureau of Investigation, Division of Forensic Sciences, P.O. Box 370808, Decatur, GA 30037*

During the past year, the State of Georgia has seen an extraordinary number of death cases directly attributable to either the knowing or the unknowing ingestion of ethylene glycol. Ethylene glycol was analyzed using a previously reported procedure from our laboratory.

Case #1 involved a white male, age 76, who drank ethylene in order to avoid prison and upon his death showed a blood level of 0.45 g/L ethylene glycol. Case number 2 was a 45 year old white male who died of an intracranial hemorrhage probably as result of multiple falls after imbibing ethylene glycol. His ethylene glycol level was found to be 0.4 g/L. The third death involving ethylene glycol was a white male, age 48, who allegedly obtained antifreeze from his mother's residence, but was not observed drinking it. However, subsequent analysis showed the presence of ethylene glycol in his blood at a level of 0.88 g/L, 1.2 g/kg in the liver and 1.8 g/kg in the kidney. A 58 year old white male with an altered mental status presented himself at a hospital whereupon he died a few hours later. Autopsy showed the presence of oxalate crystals in his kidneys and subsequent analysis showed a blood level of 3.2 g/L of ethylene glycol. A 37 year old white male was most likely poisoned by ethylene glycol after fathering his second child by one of his daughters. This individual was found to have 2.4 g/L ethylene glycol in his blood. In yet another ethylene glycol poisoning case, a 48 year old white male had a history of attempted suicide due to antifreeze and was found dead in his car. Analysis of the blood showed a postmortem blood ethylene glycol level of 1.5 g/L. A white male, age 31, who died 6 years earlier under rather mysterious circumstances, was exhumed after it was determined that his wife's current live-in boyfriend had died of ethylene glycol poisoning. It was found that this individual, a 32 year old white male, had 0.27 g/L ethylene glycol in his blood. The liver from the exhumed body of the husband was subsequently examined and was found to also have ethylene glycol at a level of 1.6 g/kg. To our knowledge, this is the first reported case of ethylene glycol having been found in the tissues of an exhumed body.

Key words: Ethylene glycol, Poisoning, Postmortem toxicology

30

Foxy, a Designer Tryptamine Hallucinogen

Robert Meatherall*[1] *and Pankaj Sharma[2]. [1]Laboratory Medicine, St. Boniface General Hospital, Winnipeg, Canada;[2] Emergency Department, Victoria General Hospital, Winnipeg, Canada.*

Foxy is a colloquial name given to 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT). It is a hallucinogen and a mild euphoric drug. Structurally, it is an analogue of the better know hallucinogen N,N-dimethyltryptamine.

A 21 year old Caucasian man presented to the emergency department about 1.5 hours after ingesting a pill called Foxy. He bought the pill for $10 to get high. Within an hour of ingestion he felt weird. He started seeing unfamiliar symbols on the wall and could not move his limbs. There was no nausea, pain or visual deficit. His physical examination was unremarkable. The effects subsided 3.5 hours after ingesting the pill. Urine was collected for a general drug screen prior to his discharge from hospital.

A drug screen was performed using a combination of immunoassays, gas chromatography and gas chromatography – mass spectrometry. The urine was negative for alcohols. Positive screening tests were obtained for cannabis and opiates. GC-MS confirmations gave THC-COOH = 44 ng/mL, codeine = 28 μg/mL and morphine = .048 μg/mL on hydrolyzed urine samples. GC-MS examination of the urine basic extract showed codeine and 3 other peaks. The largest peak had a retention time similar to desipramine; it was identified by its mass spectrum as 5-MeO-DIPT. An earlier eluting peak was presumptively identified as the N-desisopropyl metabolite, 5-methoxy-N-isopropyltryptamine. Also, a later eluting peak was presumptively identified as the N-oxide metabolite, 5-Methoxy-N,N-diisopropyltryptamine-N'-oxide.

Authentic 5-MeO-DIPT was obtained from Health Protection Branch, Health Canada, Winnipeg. A stock 1000 μg/mL methanolic solution was used to spike urine standards between 0.5 and 4 μg/mL. The internal standard was para-chlorodisopyramide. One mL of alkalinized urine was extracted into 6 mL of MTBE/CH_2Cl_2 (2:1). The organic was transferred and evaporated into 100 μL of 0.1 N HCl. Acetonitrile (50 μL) was added and the mixture washed with hexane. After alkalization, the aqueous phase was extracted with 2 mL of CH_2Cl_2. The organic was reduced to 50 μL and 1 μL injected on the GC-MS. A Finnigan ITS40 ion trap was used in the electron impact ionization mode. GC separation was done on a 15 m x 0.25 mm x 0.25 μm film DB-1 capillary column with the oven temperature programmed from 80°C to 290 °C. The concentration of 5-MeO-DIPT in the urine was 1.7 μg/mL.

Another major 5-MeO-DIPT metabolite, 5-methoxy-indoleacetic acid = 1.3 μg/mL was measured in the urine. This metabolite was recovered from an acidified extract. The carboxylic acid was ethylated to facilitate quantitation by GC-MS.

Key words: Foxy, 5-MeO-DIPT, Hallucinogen

31

Significance of Cyclobenzaprine in Death Investigation and Impaired Driving Cases in Washington State

Ann Marie Gordon* *and Barry K. Logan, Washington State Toxicology Lab, 2203 Airport Way S.,Seattle WA 98134*

Cyclobenzaprine (Flexeril®) is a tricyclic compound structurally similar to amitriptyline, and is prescribed as centrally acting skeletal muscle relaxant. It is recommended for short term treatment of muscle spasm associated with acute, painful musculoskeletal conditions. It has a half-life of 1-3 days and is highly protein bound. The usual dosage is 10 mg, 3 times per day with a range of 20 to 40 mg per day. Whilst currently a prescription medication, the manufacturer is seeking to have this drug made available over-the counter. Therapeutic blood concentrations range from 0.003-0.036 mg/L. Fatal levels have been reported in excess of 0.4 mg/L. The drug may exhibit post mortem redistribution.

Cyclobenzaprine is a central nervous system depressant and can potentially interact with alcohol, barbiturates and other CNS depressants. Issues associated with its toxicity and psychomotor effects frequently arise in both death investigation and impaired driving cases. We reviewed cases for a 5 ½ year period in which cyclobenzaprine was a toxicological finding. Cyclobenzaprine was isolated in the basic fraction of our routine drug screen, using butyl chloride as the solvent. Analysis was performed by GCMS. The LOD was 0.01 mg/L, and the limits of linearity were 0.025 - 3.0 mg/L.

Cyclobenzaprine was present in 148 post mortem cases (approximately 1% of the cases where cyclobenzaprine would have been detected had it been present). All cases analyzed were blood, 70% were identified as peripheral blood and the remainder was either central or unreported. The average subject age was 49 years, and 50% were male. The drug concentrations were from < 0.025 - 10.7 mg/L. Cyclobenzaprine exceeded therapeutic levels, i.e. > 0.05 mg/L, in 49% of the cases, and exceeded of 0.4 mg/L, the reported threshold for lethality, in 7% (n=11). Ethanol was a concurrent finding in 22% of the cases, and 93% had other psychoactive drugs present. Most typically these were narcotic analgesics, benzodiazepines and selective serotonin reuptake inhibitors. Very few decedents (approximately 6%) had illicit drugs present.

Cyclobenzaprine was found in blood samples from 34 drivers (approximately 0.6% of drivers for whom cyclobenzaprine would have been detected), whose average age was 40, and 53% of whom were males. Half of these drivers were tested as a result of their involvement in traffic accidents. Their blood concentrations ranged from < 0.025 - 0.24 mg/L. Of these cases, only 11% had cyclobenzaprine concentrations > 0.05 mg/L. Fifteen per cent of the drivers were positive for ethanol and all had other psychoactive drugs present.

In summary, cyclobenzaprine is a frequent finding in both death investigation and impaired driving cases. Interpretation of its significance is often difficult due to the presence of other psychoactive drugs. In light of the efforts to make this available as an over-the-counter medication, we reviewed the findings of cyclobenzaprine occurrence in our cases to evaluate potential risks in overdoses and driving under the influence.

Key words: Cyclobenzaprine, Driving, Postmortem toxicology

32

Utility of Vitreous Humor in Investigations of Heroin related Deaths

Julia M. Pearson* *and Joseph J. Saady, Virginia Division of Forensic Science, Richmond, VA*

Emergency department trends from the Drug Abuse Warning Network (DAWN) show that an increasing trend (+ 15%) in heroin mentions from 1999 – 2000, while trends from mortality data (DAWN) are more ambiguous. A new analytical procedure using hydroxyl-amine to form oxime derivatives of the keto-opiates, followed by SPE and TMS derivitization allows for the simultaneous quantification/confirmation of codeine, morphine, 6-mono-acetylmorphine (6MAM), oxycodone, hydrocodone, oxymorphone and hydromorphone. This procedure requires only 2 mL sample volume. Standard curves were linear over the concentration range of 0.01-0.60 mg/L for each opiate. The limit of quantitation was 0.01 mg/L. We find that the examination of vitreous fluid can extend the detectability of 6MAM, and review the cause of death in relation to the free blood morphine: vitreous 6MAM. Free morphine concentrations ranged from 0.03-0.55 mg/L in blood and 0.00-0.15 mg/L in vitreous. 6MAM concentrations ranged from 0.00-0.04 mg/L in blood and 0.01-0.21 mg/L in vitreous. Furthermore, by examining vitreous for the presence of 6MAM, we were able to identify 54% more cases involving heroin than if only iliac blood was analyzed.

In blood, heroin is rapidly hydrolyzed to morphine. This process appears slowed or reduced in vitreous as evidenced by morphine:6MAM ratios (n=22 cases). The ratio of morphine:6MAM was 15 in blood and 1.5 in vitreous. In 38% of cases, vitreous 6MAM concentrations were equal to or greater than vitreous morphine concentrations. In addition, vitreous 6MAM concentrations were 6 times greater than blood 6MAM concentrations.

In summary, analysis of vitreous greatly increases the detectability of 6MAM and assists in the interpretation of morphine levels in death investigations, particularly in cases involving delayed opiate toxicity.

Key words: Morphine, Heroin, Vitreous humor

33

PMA Overdose Case: Examination of Blood, Brain and Hair

Kathryn S. Kalasinsky[*1]*, Marcie M. Dixon*[1]*, and Stephen J. Kish*[2]*. [1]Armed Forces Institute of Pathology, Division of Forensic Toxicology, 1413 Research Blvd., Rockville, MD 20850; [2]Centre for Addiction and Mental Health, Human Neurochemical Pathology Laboratory, 250 College St., Toronto, Ontario, Canada M5T 1R8*

Blood, brain and hair of an 18-year-old Canadian male from an apparent drug overdose case were examined. Solid Phase extraction was employed using UCT Clean Screen columns with the UCT recommend amphetamine procedure and derivatization with HFBA, followed by GC/MS analysis using a Varian Saturn 2000 ion trap system with a DB-5 GC column. The ions monitored for PMA were 121,148 and 91 m/z, and for PMMA were 254, 148, and 121 m/z. The LOD/LOQ for the various matrices was 0.02 ng/mg for hair, 0.02 mg/kg for brain and 0.002 mg/L for blood. The hair was washed and prepared by acid digest prior to extraction. The blood (1ml) was positive for PMA, 0.81mg/L, PMMA, 0.32 mg/L, and benzoylecgonine, 0.77mg/L. The brain (100mg) was positive for PMA, 3.39 mg/kg, and PMMA, 0.96mg/kg, only. Previous literature findings for postmortem PMA values range from 0.2–4.9 mg/L for blood, and from 2.8–6.6 mg/kg for brain. The hair was plucked from the scalp and the root bulbs analyzed as well as segments approximating one month's hair growth for each segment from the scalp to the distal end. Root bulb analysis has been shown to be useful in determining the drugs in the body at the time of death.

The root bulbs (2.7mg) were positive for PMA, 18.01 ng/mg, and PMMA, 8.44 ng/mg. All of the hair segments (sample weights varying from 2 – 13mg) were positive primarily for PMA, 5.66–7.94 ng/mg. Lower amounts of PMMA, 1.46–3.02 ng/mg, and MDMA, 0.37–7.78ng/mg, were also present in all of the segments. Some of the segments were also positive for small amounts of MDA, 0.49-1.03 ng/mg, and amphetamine, 0.43–0.44 ng/mg. The most likely explanation for these forensic findings is that the subject was a chronic "ecstasy" user who had unknowingly ingested PMA sold as "ecstasy", both chronically (approximately five months duration) and acutely, with the last dose of the highly toxic drug causing death. These data also reinforce the public health danger of obtaining tablets purported to contain ecstasy. This work was supported by US NIH NIDA 7182.

Key words: PMA, Ecstasy, Blood/brain/hair analysis

34

Isopropanol: A Putrefactive Product in Deaths Due to Drowning

Teri L Martin* *and Brendon R. Lalonde, Centre of Forensic Sciences, 25 Grosvenor St. Toronto, Ontario, Canada, M7A 2G8*

Isopropanol and acetone may be detected in the blood and urine as a result of ketoacidosis, fasting, or the ingestion of either of these substances. Analyses for isopropanol, ethanol, methanol, acetaldyhde, acetone and n-propanol are routine toxicological examinations performed in our laboratory. Although the postmortem production of ethanol and *n*-propanol has been well-documented in the forensic literature, isopropanol is not typically considered to be a common putrefactive product. In four recent cases of death due to drowning, however, evidence for the postmortem formation of isopropanol was obtained.

Decomposition was evident in each of the four drowning cases, and the presence of *n*-propanol served as an indicator of putrefaction. Isopropanol was detected and quantified in the blood of the individuals at concentrations ranging from 6 mg/100 mL to 22 mg/100 mL. There was no history of isopropanol consumption in any of the cases and acetone concentrations were less than 2 mg/100 mL in all four cases, suggesting that the isopropanol detected did not arise through the metabolism of acetone or through the ingestion of isopropanol. A subsequent review of the literature revealed corroborating evidence for the postmortem production of isopropanol in drowning victims (Jones, 1985. Proc. 22nd Annual Meeting of TIAFT. Rigi-Kaltbad, Switzerland. August 26-29. pp. 67-73).

Isopropanol and acetone concentrations from the four drowning cases were compared to 5,879 other specimens that were analyzed for ethanol and other volatiles from January 1, 2001 to December 31, 2001. Of these, only 38 had evidence of both putrefaction and measurable concentrations of isopropanol. In contrast to the four drowning cases, which were characterized by high isopropanol concentrations and negligible acetone concentrations, the majority of the other specimens displayed acetone concentrations that exceeded the detected isopropanol concentrations.

The results of this study indicate that where isopropanol is present in a death due to drowning, a possible explanation is the postmortem neogenesis of isopropanol.

Key words: Isopropranol, Drowning, Putrefaction

35

Comparison and Evaluation of DRI® Methamphetamine, DRI® Ecstasy, Abuscreen® Online and a Modified Abuscreen® Online Screening Immunoassays for the Detection of AMP, MTH, MDA and MDMA in Urine

Peter R. Stout[1], Russell Wiegand[2] and Kevin L. Klette[2]. [1]Aegis Sciences Corp. 345 Hill Ave. Nashville TN 37210 [2]Navy Drug Screening Laboratory, PO Box 113, Bldg. H-2033, Naval Air Station, Jacksonville FL, 32212

The performances of four immunoassays (DRI® amphetamines, DRI® ecstasy, Abuscreen® Online amphetamines and a modified Abuscreen® Online amphetamines) were evaluated for control failure rates, sensitivity and specificity for amphetamine (AMP), methamphetamine (MTH), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphet-amine (MDMA). The two DRI® reagents and the Online reagents were run according to manufacturer specifications using a Hitachi Modular DDP system. The modified Online reagent was calibrated with MDMA and had 16 mM sodium periodate added to the R2 reagent. These assays were run on approximately 27,000 human urine samples and 7,000 control urine samples prepared at 350 ng/ml and 674 ng/ml over the course of 8 productions days. All assays were calibrated using a single point, qualitative cutoff standard with the manufacturer recommended compound at the department of defense cutoff (500 ng/ml). A solid phase extraction method was used to prepare samples after treatment with sodium periodate and isolate AMP, MTH, MDA and MDMA from urine. Extracts were derivitized using heptafluorobutyric anhydride (HFBA). Derivitized samples were analyzed by gas chromatography/mass spectrometry (GC/MS). A separate aliquot of each sample was qualitatively analyzed for the presence of ephedrine and pseudoephedrine using the same extraction and derivitization without the periodate pretreatment. Control performance for the manufacturer recommended assays was excellent with a maximum qualitative control failure rate of 2.03% (where qualitative positive controls failed to perform positive or qualitative negative controls failed to perform negative). The modified Online reagent demonstrated poor control performance with a maximum failure rate of 38.3% and showed no improved MDMA sensitivity (determined by an increase in detecting samples containing MDMA) when compared to the Online reagent; the confirmation rate was improved when compared to the Online reagent (20% compared to 8%). The DRI® Ecstasy reagent provided improved sensitivity for MDMA as compared to the Online reagent with approximately 23% more samples screening and confirming positive for MDMA and a confirmation rate of approximately 90%. The DRI® methamphetamine reagent had a low confirmation rate (6% or less) and produced numerous positives for samples with only ephedrine or pseudoephedrine present.

Key words: Immunoassay, Ecstasy, Amphetamines

36

DNA Electronic Microarray Detection for Pharmacogenomics – Genotyping CYP-450 Mutations as Molecular Autopsy for Certifying Drug Related Toxicity

Steven H. Y. Wong*[1,2], *Paul J. Jannetto*[1,2], *Elvan Sahin*[1,2], *Chuck Schur*[1], *Jimmy Crockett*[1,3], *John A. Ndon*[3], *Robert C. Mucic*[4], *Michael Catania*[4], *and Tim Tiemann*[4]. [1] *Path. Dept., Medical College of Wisconsin,* [2]*Milwaukee County Medical Examiners' Office,* [3]*University of Wisconsin – Milwaukee, Milwaukee, WI.; and* [4]*Motorola Life Sciences , Pasadena, CA, U.S.A.*

In the current Postgenomics era, molecular diagnostic may provide genetic information: in clinical diagnosis of diseases through the use of genetic biomarkers., in the drug discovery process by stratifying patients' genetics with the optimal drug and dosage., and in the emerging use of molecular diagnosis as molecular autopsy in forensic pathology/toxicology. This was demonstrated in the molecular analysis of *SCN5A* defects in sudden infant death syndrome (Ackerman et al. *JAMA* 2001;286:2264-9). Another emerging application of Molecular Autopsy is the use of pharmacogenomics for understanding adverse drug reaction as a basis for certifying drug related toxicity. This study assessed the potential clinical efficacy of a DNA electronic microarray detection - eSensor™ DNA biochip for genotyping selected mutations of CYP 2D6 genes encoding cytochrome the drug metabolizing enzymes. After the DNA extraction using whole blood, the desired region of genomic DNA containing the polymorphism - DNA base substitution/deletion, was amplified by PCR with UTP and UNG. The resulting PCR product was digested with Lambda Exonuclease to form single-stranded DNA. This was injected, with the signaling probes and assay buffer, onto an eSensor™ DNA biochip cartridge, without further purification or washing steps. If present, the target amplicon DNA hybridized to the complementary capture and signaling probes, bringing the specific electrochemical labels on the signaling probe near the electrode surface. The eSensor™ utilized alternating current voltammetry to detect the electrochemical signals from each electrode on the eSensor™ cartridge. This data was then used to determine which of the cytochrome P450 polymorphisms were present in the target DNA. Both the wild-type and variant signaling probes were labeled differently, allowing the eSensor™ system to determine whether the wild-type or variant sequences of a specific cytochrome P450 gene were present. This protocol, performed within about 8 hours, was used to genotype several *CYP 2D6* alleles for 20 samples. Results for *CYP 2D6*4* were compared to previously established results by realtime PCR using the Lightcycler system. Both protocols showed 2 homozygous, 5 heterozygous and 13 wild-type for *CYP2D6*4*. Thus, the eSensor™ DNA microarray may be potentially used for genotyping *CYP 2D6*4* as an adjunct for certifying drug toxicity.

Key words: Molecular autopsy, Pharmacogenomics, DNA electronic microarray, *CYP 2D6*4*

37

Pharmacogenomics as Molecular Autopsy – Genotyping *CYP 450 2D6*3/*4/*5* for Certifying Antidepressants and Selected Opioids related Toxicities

*Steven H. Y. Wong[1,2,3,4], **Elvan Sahin*** [1,3], Paul J. Jannetto[1,3,4], Michael A. Wagner[1,3], Chuck Schur[1], Jimmy Crockett[1,4], John A. Udon[4], Robert Risinger[1,2], Susan B. Gock[1,3], and Jeffrey M. Jentzen[1,3]. [1]Path. Dept., [2]Psych. & Behavioral Med. Dept., Med. Coll. WI., [3]Milwaukee(Mil) Co. Med. Examiners' Off., and [4]Univ. WI–Mil., Mil., WI, U.S.A.*

Molecular diagnostic may enable individualized drug therapy by stratifying patient's genetics with the optimal drug and dosage as in antidepressant therapy, and may function as molecular autopsy in the forensic pathology/toxicology as shown by genotyping *SCN5A* defects in sudden infant death syndrome.(Ackerman et al. JAMA 2001;286: 2264-9). Molecular autopsy may be defined as the application of molecular diagnostics – pharmacogenomics and/or proteomics markers - as an adjunct for interpretation of the cause and/or manner of death. Another emerging application of Molecular Autopsy is the use of pharmacogenomics (PG) as an adjunct for understanding adverse drug reaction as a basis for certifying drug related toxicity, supplementing autopsy findings, case and medication histories, toxicological analysis, and death scene investigations. This study examined the applications of PG - genotyping *CYP 2D6*3/*4/*5* for certifying toxicities related to drugs metabolized by CYP 2D6 enzymes: antidepressants, and selected opioids – codeine and hydrocodone. Currently, a clinical study is being planned to apply PG for genotype-based antidepressant therapy. For the Molecular Autopsy study, genotyping was performed, preceded by DNA extraction of whole blood, by conventional and/or realtime PCRs for *CYP 2D6*3/*4*, and long PCR for *CYP 2D6*5*. PG and toxicology results were:

Case	CYP 2D6 *3/*4/*5	Drugs	Conc. mg/L	Other drugs	COD	MOD
1	WT/HM/WT	Ami/Nor	1.5/2.2	Diaz./Methadone (0.7)	MDT	Accident
2	WT/HT/WT	Codeine	0.01	Oxycodone/Diaz.	Oxycod.OD	Accident
3	WT/HT/WT	Venlafaxine	0.19	Alcohol/Methadone	Sudd. Card.	Natural
4	WT/HT/WT	Venlafaxine	0.29	Cycloben./Diaz./Oxycod.	MDT	Suicide
5	WT/WT/WT	Hydrocodone	0.04	Cocaine/BE/Fentanyl/Oxycodone	MDT	Accident
6	WT/WT/WT	Hydrocodone	0.06	Cital./Carisoprodol-Mepro./Oxy.	MDT	Accident
7	WT/WT/WT	Ami/Nor	0.55/0.39	Alcohol/Methadone	MDT	Accident
8	WT/WT/WT	Ami/Nor	0.63/ND	Diaz./Li/Methadone	Methad.OD	Accident
9	WT/WT/WT	Dox/N-Dox	0.72/0.07	Alcohol/Methadone/Clona./Alpra.	MDT	Accident
10	WT/WT/WT	Ami/Nor	0.92/ND	Oxycodone	MDT	Accident
11	WT/WT/WT	Hydrocodone	1.01	Aceta./Diaz./Diphen./Oxy	MDT	Suicide
12	WT/WT/WT	Nortriptyline	13.57	Oxycodone	MDT	Undet.

The abbreviations are: WT, wide-type., HT, heterozygous., HM, homozygous., ND, non-detected, and MDT, mixed drug toxicity.

(continued)

The majority were certified as mixed drug toxicities. Case 1 was a *CYP 2D6*4* HM - without the CYP 2D6 enzyme to metabolize both tricyclics and methadone. Cases 2, 3 and 4 were *CYP 2D6*4* HT corresponding to reduced enzyme activities, with therapeutic concentrations of venlafaxine and codeine but elevated concentrations of oxycodone- 2 (case 2) and 0.84mg/L (case 4), and 0.5 mg/L of methadone (case 3). Cases 5 to 12 were identified as WT. For decedents with either HM or HT for CYP 2D6, they would be more likely to experience toxicity on therapeutic doses. The overall drug toxicity and death might be more likely to be certified as accidental. By interpreting the PG results with toxicology, autopsy findings and death scene investigations, PG, as molecular autopsy, served as an adjunct to explain drug related toxicity in some of the cases.

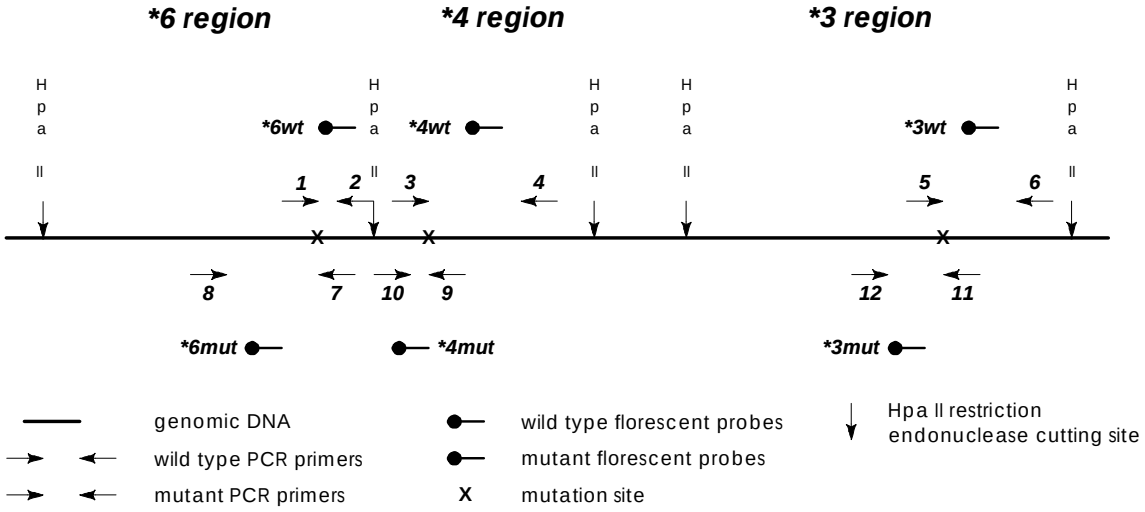
Key words: Pharmacogenomics, Molecular autopsy, CYP 2D6, Antidepressants, Opioids

38

The Potential of a Molecular Autopsy – Multiplexed CYP 2D6 *3 *4 and *6 Polymorphism by Real Time PCR

Michael A. Wagner* *Sameer Sakallah Department of Health and Human Services Toxicology and Molecular Diagnostic Laboratory 6 Hazen Dr. Concord, NH. U.S.A.*

Genotypic association studies of an individual's drug metabolism may establish that individual's pharmacogenetic profile. Metabolism differences in therapeutic and abuse drugs may contribute to a toxic/lethal outcome by altering the pharmacologically active drug/metabolite concentration relative to the dose. Linder et al Clin. Chem. 1997; 43:2 254-266 indicated three major phenotypes exist: ultraextensive, extensive, and poor drug metabolizer. The gene for cytochrome P450 2D6 (CYP 2D6) encodes the enzyme desbrisoquine 4-hydroxylase which metabolizes more than 25 typically prescribed medications. In addition, CYP 2D6 metabolizes certain synthetic drugs of abuse. This gene is polymorphic containing as many as 50 alleles. 5-10 % of the Caucasian population contains one or more of these alleles, while 17 % of some ethnic populations may be effected. This study used whole blood source purified genomic DNA from seven individuals with the alleles *3, *4, and *5. Here a new method for detecting the *3 and *4 allele was developed, as well as, a new allele *6. DNA digestions with the Hpa II restriction endonuclease is designed to cut the region harboring the sites for *3, *4 and *6 mutations as outlined in the diagram. The DNA digestion aliquot is amplified by PCR using allele specific primers. Three Taqman probes (FAM, HEX, or ROX florescent dyes - one for each wild type or mutant allele) can be used for the detection of the amplification products via the SmartCycler real time PCR system.



Digesting the genomic DNA with Hpa II will eliminate the interference of PCR primers between each other for different mutations. Concurrently, this method enables the reactions to be combined into 2 multiplex reactions: one for detecting any or all of these mutations, and the other for detecting the wild type alleles. The implementation of this molecular assay in combination with toxicology assays will enable the toxicologist to establish a pharmacogenetic association with the appropriate drug.

Key words: Multiplex, PCR, CYP 2D6

39

Pharmacokinetics and Excretion of Gamma-hydroxybutyrate (GHB) in Healthy Subjects

Rudolf Brenneisen[1], Mahmoud A. ElSohly[2], Timothy P. Murphy[2], David E. Watson[2], Stefan Russmann[3], Joe Passarelli[4],and ***Salvatore J. Salamone****[5]. *[1]University of Bern, Dep. of Clinical Research, Bern, Switzerland; [2]ElSohly Laboratories Inc., Oxford, MS, U.S.A.; [3]University of Bern, Institute of Clinical Pharmacology, Bern, Switzerland; [4]Roche Diagnostics Corp., Indianapolis, IN, U.S.A.; [5]Orasure Technologies Inc., Bethlehem, PA, U.S.A.*

In recent years, an increasing use of GHB as dance party, lifestyle and date-rape drug has been observed in both Europe and the United States. It was the aim of this IRB approved study to acquire pharmacokinetic profiles and measure excretion rates and detection times in GHB-naïve volunteers after a single oral dose of GHB.

Four male and 4 female healthy subjects with no GHB experience received a single 25-mg dose of GHB per kg body weight orally. Blood and oral fluid samples were collected up to 480 min postdosing, and urine was collected up to 24 h at specific times. In addition, vital functions were monitored and psychological as well as somatic side-effects reported by using visual analog scales. The GHB concentrations were determined by GC/MS. The extraction procedure was based on acid conversion of GHB to gamma-butyrolactone in the presence of gamma-valerolactone as internal standard followed by chloroform extraction. Analysis was then carried out in the SIM mode with a LOQ of 0.5 and 0.2 μg/mL for blood and urine, respectively.

GHB plasma peaks of 39.9 ± 8.3 μg/mL (mean ± SEM) occurred 37.5 ± 10.9 min after application. The terminal plasma half-life ($t_{1/2z}$) was 31.1 ± 2.5 min, the total clearance (CL_{tot}) 1276 ± 308 mL/min and the distribution volume (V_z) 61.7 ± 19.8 L. In urine, the highest levels of GHB were detected at 30 and 60 min at 175 ± 70.1 and 230 ± 86.3 μg/mL, respectively. Only 1 ± 0.2 % of the dose was recovered, resulting in a detection window of 240 to 720 min. In oral fluid, GHB could be detected up to 150 min post dosing, with peak concentrations of 203 ± 92.4 μg/mL in the 10-min samples.

Common side-effects were confusion, sleepiness, and dizziness. Relevant psychotropic effects and a change of vital functions were not observed.

In conclusion, GHB is extensively metabolized and rapidly eliminated in urine and oral fluid. Detection times are short and efforts should be made to collect samples in as short of time as possible from the time of ingestion.

Key words: GHB, pharmacokinetics, GC/MS

39 continued

DETAILS OF THE EXTRACTION/ANALYSIS PROCEDURE

Extraction Procedure:

Urine

2.0 mL of urine were measured with a 5 mL pipette and transferred to a 15 mL centrifuge tube. Calibration tubes were prepared by adding to each tube 50 μL of internal standard solution (gamma-valero-lactone, 1.0 mg/mL). An appropriate amount of GHB standard solution (1.0mg/mL) was added with 50 μL of internal standard solution to 2 mL blank urine for each calibrator. To each tube was added 0.5 mL of 20% trifluoroacetic acid and each tube was vortexed. The tubes were capped and placed in an oven at 75°C for 1 hr. The tubes were then removed from the oven and allowed to cool down to room temperature. 0.55 mL of 2 NaOH was added to each tube to adjust the pH to 6.5. 3 mL of chloroform was added to each tube. The tube was capped and shaken for 1 minute. Approximately 0.5 mL of the organic layer (bottom) was transferred to a GC vial for injection (for dilute samples the chloroform layer could be concentrated and the concentrate transferred to a GC vial insert for injection).

Plasma

The procedure was the same as in urine except that 0.5 mL plasma was used which was diluted with 1.5 mL deionized water before proceeding with the extraction process.

Saliva

The extraction of saliva samples was carried out by measuring 1 mL of the saliva/diluent from the collection device or whatever volume collected after centrifugation of the device and dilution with enough volume of deionized water to 2 mL. The mixture was then extracted following the same procedure as urine.

Analysis Conditions

An HP-5890 gas chromatograph interfaced with an HP-5970B MSD was used. GC conditions: 25 M x 0.2 mm (0.33 μ film) DB-5 MS column operated from 60°C (1 min) to 90°C at 10°C/min. The temperature was then raised to 270°C at 35°C/min for 1 min. Oven equilibrium time was 0.5 min. Helium carrier was used at a velocity of 38 cm/sec. The electron multiplier voltage was the same as tune value. 2 μL of each sample were injected. Ions were monitored at 42 (Q), 56 and 86 (Group I) for GHB; and 56 (Q), 41 and 85 for internal standard. The retention times for GHB and internal standard were 4.35 min and 4.80 min, respectively.

ABSTRACTS

POSTER PRESENTATIONS

P1

ICP-MS Uncovers Life Threatening Mercury + Cadmium Intoxication with Negative GC/MS and LC/MS Blood Screens for Rat Poison

***Ernest D. Lykissa*[1]**, *Carlos A. Gonzalez*[1], *Imad Kaffity*[2], *and Roger Peterson*[2]. [1]*Expertox Laboratories, Deer Park, TX, U.S.A.;* [2]*Mineral King Laboratories, Tulare District Hospital, Tulare, CA, U.S.A.*

Mercury, upon its absorption in a biological system, results is cell membrane damage, CNS toxicity, teratogenicity, and the divalent cation form of mercury has high-binding affinity to thiol or sulfhydryl protein groups, thus resulting in the indiscriminate denaturation of proteins. Cadmium, is a silver-white metal highly resistant to corrosion. Cadmium toxicity is characterized by severe GI involvement with subsequent renal damage.

We were asked to analyze a young mother's blood, with history of methamphetamine illicit manufacture and abuse, for the presence of rat poison given to her by her daughter as green sauce in tacos for dinner. The mother ate some of the tacos but didn't like the taste, so she fed the left-overs to the dog who died shortly after. The mother was hospitalized in suffering from severe symptoms of poisoning, and she is presently on 100% oxygen suffering from severe kidney failure, and respiratory distress, with very poor prognosis. GC/MS and LC/MS analyses of the blood did not result in any significant findings for the anticoagulants Brodifacoum, Warfarin, Diphacinone, Bromodiolone, Chlorophacinone. They were also negative for Strychnine. The levels of mercury, cadmium, lead, arsenic, chromium, nickel, cobalt, zinc and copper were measured in the same blood specimen by ICP-MS to eliminate the possibility of arsenicals or other heavy metals. The resulting toxic elevations in the blood of Mercury 580 :g/L (Toxic > 100 :g/L) and Cadmium 56.8 :g/L (Toxic > 5.0 :g/L) were subsequently confirmed in the same patients' urine collected six days later, we obtained urine Mercury 850 :g/L (Toxic > 150 :g/L, Lethal > 800 :g/L), and urine Cadmium 1850 :g/L (Toxic > 100 :g/L) while we eliminated all probable causes of contamination of the specimens tested. The source of the intoxication was attributed to a mixture of expended methamphetamine manufacturing waste. The findings in this particular case clearly demonstrate the forensic attributes of the ICP-MS technology.

Key words: ICP-MS, Mercury, Cadmium.

P2

Improved Rapid and Sensitive Analysis of THC and Metabolites in Whole Blood by Disposable Pipette Extraction

William E. Brewer* *and Brandi L. Clelland. Clemson Veterinary Diagnostic Center, Columbia, SC*

A rapid extraction method has been developed that is referred to as disposable pipette extraction (DPX). DPX uses a pipette tip that is fitted with a frit, and it contains loosely packed sorbent material for performing solid-phase extraction (SPE). Sample is drawn into the tip via pipette, and after an equilibration time, the sample is delivered to waste. After a rapid wash step, adsorbed analyte is eluted with less than 1 mL of organic solvent. Some of the advantages of DPX are that a time-consuming conditioning step and large solvent volumes are not required, and DPX has been shown to be almost ten times faster than conventional SPE. Recently, a 5 mL DPX tip has been manufactured to accommodate larger sample volumes to improve analytical sensitivity.

The analysis of tetrahydrocannabinol (THC) and its hydroxy and carboxy metabolites in blood is tedious and time-consuming by conventional methods. DPX has been successfully applied to the analysis of THC, OH-THC and COOH-THC in whole blood. Spiked whole blood (1.0 mL bovine whole blood) was first treated with 2.0 mL acetonitrile, and the supernatant was transferred to a clean test tube following centrifugation. Water (2.0 mL) was added to the supernatant and the sample was extracted by DPX. Only 0.5 mL of wash solvent (33% acetonitrile in water) and 1.0 mL of elution solvent (50% ethyl acetate in acetonitrile) was used for the extraction. The total extraction time (after protein precipitation) took less than 3 minutes per sample. The extracts were subsequently derivatized with bis(trimethylsilyl)trifluoro-acetamide, and analyzed by GC/MS (Hewlett-Packard 6890 GC equipped with 5973 MSD; GC column was 30 m Rtx-5ms (Restek) with 0.25 mm ID and 0.2 um film) with selected ion monitoring. (The target ions were m/z = 386, 389, 371, 374, 473, and 476 u for THC, d_3-THC, OH-THC, d_3-OH-THC, COOH-THC, and d_3-COOH-THC, respectively).

Based solely on a signal-to-noise ratio, the limit of detection (LOD) for THC, OH-THC and COOH-THC were 0.25, 0.18 and 2.0 ng/mL, respectively. However, the criteria for the LOD to have 2 qualifier ions that have ion ratios within 20% of their expected ratios leads to LOD levels of approximately 0.5, 0.5 and 2.0 ng/mL, respectively. The higher LOD levels were due to an interfering peak associated with m/z = 371 u for THC, lack of prominent fragmentation ions for OH-THC, and low recovery for COOH-THC (see below). Lower LOD levels may be expected by using an alternative derivatization method. The limit of quantitation (LOQ, which was determined by calculating the concentration of a standard from a linear plot (R2 greater than 0.99 for all three analytes) to be within 20% of the expected concentration) was also calculated to be 0.5, 0.5 and 2.0 ng/mL for THC, OH-THC and COOH-THC, respectively. The reproducibility (%C.V., N = 10), determined from the ratio of the base peak of the analyte to the base peak of the internal standard, was 0.90% for THC at 5 ng/mL, 0.66% for OH-THC at 1 ng/mL, and 0.51% for COOH-THC at 10 ng/mL (from the first extraction--aqueous fraction). No issues of carryover were observed for any of the analyses.

(continued)

The recoveries (including the protein precipitation) for THC, OH-THC and COOH-THC were 53%, 60% and 26%, respectively. The sample solution was re-extracted (with the same corresponding DPX tip) after addition of HCl to improve the recoveries of COOH-THC. This second extraction leads to an additional recovery of approximately 30% (56% total). This second additional extraction was not necessary for the analysis of COOH-THC for most forensic applications because the immunoassay cutoff is generally greater than 10 ng/mL.

Key words: Disposable pipette extraction, Solid-phase extraction, GC/MS

P3

Implementing a Regulatory-Compliant Electronic Records Strategy in the Clinical Chemistry Laboratory

Robert J. Whitehead*, *Melissa A. Ivie. ChemWare Inc., Raleigh, NC, U.S.A.*

We review the current status of compliance standards and technologies with respect to the capture, storage and transmission of electronic records, focusing on future directions impacting clinical and toxicology laboratory data management. Only in the past few years have the regulators, judiciary and computing industry all come into alignment, dramatically accelerating advancements in information systems and paving the way for the inevitable paperless laboratory.

The efforts of members of the pharmaceutical industry, dating back to 1991, led to the FDA's creation of a Task Force on Electronic Identification/Signatures, and eventually to the promulgation of the 1997 *Final Rule on Electronic Records/Electronic Signatures* (21 CFR Part 11). The combination of FDA acceptance of electronic records and the 1998 passing of the *Government Paperwork Elimination Act* laid the groundwork for the DOT, EPA and other regulators and data end-users. The EPA proposed its version of the regulations in the *Cross-Media Electronic Reporting and Recordkeeping Rule* (CROMERR) of 2001, currently undergoing a second round of public comment. Both SAMHSA and the DOT have long-recognized the inevitability of electronic recordkeeping, but have yet to finalize standards. The DOT created the Electronic Transmission and Storage of Drug Testing Information Federal Advisory Committee to assess currently available technology, with final recommendations expected in late 2003.

With the FDA setting the pace, it is not surprising that pharmaceutical chemists were among the first targeted by the scientific data management industry. Since 1996, all twenty-five of the top pharmaceutical companies have installed and are currently using the NuGenesis® Scientific Data Management System (NuGenesis Technologies Corporation, Westborough, MA, USA) to foster collaboration and accelerate the new drug discovery and development process. In early 2002, NuGenesis and ChemWare (Raleigh, NC, USA), a leading laboratory information management systems provider in the clinical/toxicology arena, joined together to integrate NuGenesis Vision™ with the HORIZON® LIMS. Microsoft Windows® Metafile and Oracle® database technologies are combined to unify the data from dissimilar sources into a common electronic format. Chemists use a patented "print-to-database" technology to electronically capture reports, including graphics, from any Windows-based application. Virtually any instrument can be interfaced to the system, enabling simultaneous data parsing and printing of the instrument output into the database. A vector-scalable image of the output is aggregated with pertinent metadata, then stored in a highly indexed Oracle repository. Advanced search and filter capabilities allow the data end-user to find any text string in any report, even within graphical images. A chromatogram with "benzoylecgonine" aligned vertically across a chromatogram peak, which might otherwise be printed on paper and stored in a folder in a box on the top shelf of an off-site warehouse, is located in HORIZON in a matter of seconds.

(continued)

We demonstrate how clinical chemists use HORIZON data management tools to automatically create a compound document, such as a litigation package, then apply a 21 CFR Part 11-compliant electronic signature to the report. Various return on investment metrics show significant reductions in the costs associated with report preparation and data storage. Labor is reduced from several hours per report to less than a few seconds, with corresponding savings in printing, photocopying and hardcopy data storage.

Keywords: LIMS, 21 CFR Part 11, Electronic records

P4

Liquid Chromatography - Tandem Mass Spectrometry for Clinical Drug Analysis of Rapamycin and for Forensic Toxicology

Steven H. Y. Wong*[1,2,3], *Paul J. Jannetto*[1,2,3], *Elvan Sahin*[1,2], *Jimmy Crockett*[1,3], *Susan B. Gock*[1,2], *Jeffrey M. Jentzen*[1,2], *Donald Mason*[4], *Roger A. Upham*[4], *Michael R. Morris*[5], *and Donald P. Cooper*[5]. [1]*Path. Dept., Medical College of Wisconsin,* [2]*Milwaukee County Medical Examiners' Office,* [3]*University of Wisconsin – Milwaukee, Milwaukee, WI.,* [4]*Micromass Corporation, Beverly, MA, U.S.A.., and* [5]*Micromass Corporation UK , Manchester, UK.*

Advances in mass spectrometer design, improvements in software and instrument control have greatly enhanced its application for clinical drug and toxicological analyses. This preliminary study assessed the feasibility of rapid analysis of an immunosuppresssant – rapamycin by using a liquid chromatography tandem spectrometer. It will be followed by further applications in forensic toxicological analyses. Sample preparation included protein precipitation, followed by injection of the supernatant. Sample aliquots – 100 uL of whole blood, were mixed with 200 uL of acetonitrile containing an internal standard, ascomycin. After vortex mixing, the mixtures were centrifuged for 3 minutes, followed by transferring the supernatant for HPLC injections. Chromatograhpic parameters were: injection volume - 20 uL, column – C18 (2.0 x 15 mm, Waters Xterra MS), mobile phase/gradient elution – MeOH/NH_4OAc/0.1% formic acid(1:1) to MeOH 100% at 0.1 minute, column temperature – 45 ^{0}C, flow rate – 500 uL/min, total analysis time – 2.25 min. Mass spectrometer parameters were: Micromass Quattro micro^{TM}, mode – positive ion electrospray, gas cell pressure – 3.0 e-3 mBar argon, cone – 20 V, collision energy – 19 eV, ammoniated ion of rapamycin – *m/z* 931.5 $\rightarrow$ *m/z* 864.2, and ammoniated ion of ascomycin – *m/z* 809.4$\rightarrow$ *m/z* 756.2, temperatures – source, 80 ^{0}C., and desolvation, 250 ^{0}C. Rapamcyin and ascomycin eluded at 1.29 min. Their ammoniated and the dominant product ions were monitored.

Calibration was linear from 1 to 20 ng/mL, with linear regression analysis showing $r^2 = 0.9957$. Within run analysis showed a 6.6% RSD for 60 injections of 2 ng/mL samples. Patients samples (n = 5) showed a concentration range of 3.5 to 9 ng/mL, within the expected therapeutic range of rapamycin (3 to 15 ng/mL). The ease of sample preparation, coupled with high sensitivity and specificity of tandem mass spectrometry offered a suitable analysis for low concentration drug such as rapamycin. This technique will be assessed for drug analyses in forensic toxicology.

Key words: LC/MS/MS, Immunosuppressants, Rapamycin

P5

Simple, Rapid, Sensitive, Direct Method for the Identification and Quantitation of Gammahydroxybutyric Acid (GHB) in Urine

John Vasiliades*, and Kim Ford. Toxicology Labs Inc., 4472 South 84th St., Omaha, NE 68127*

We present a rapid, sensitive, direct injection procedure for the identification and quantitation of gammahydroxybutyric acid (GHB) in urine. The advantage of this method is that no extraction, derivatization or heating step is required prior to gas chromatographic (GC) analysis. Gammahydroxybutyric acid or its salt is in dynamic equilibrium with its lactone, (GHL). We take advantage of this equilibrium for our analysis of GHB by measuring GHL. The lactone which is formed is analyzed by GC with a flame ionization detector (FID) on an alcohol column, 1.82 m x 2 mm ID glass column, 60/80 Carbopack B/ 5% Carbowax 20M (Supelco, Bellefonte, PA, 16823). Ethylene glycol (EG) is used as internal standard (IS). Urine (0.1 mL) is mixed with 0.1 ml of internal standard (EG, 444 mg/L). One microliter of sample is injected directly on the alcohol column. Gas Chromatography conditions are: injector 200^{o}C, detector 300^{o}C, column 175^{o}C, carrier gas helium with a flow rate of 30 mL/min. Retention time for GHL is 1.7 relative to EG 1.3 minutes.

Excellent linearity was observed in the 100 to 1000 mg/L range (slope = 1.29, y-intercept = 0.0036, correlation coefficient = 1.0). Within-run precision is 6.7% at 100 mg/L, 6.8% at 500 mg/L and 8.2% at 1000 mg/L. Between-run precision is 11.2 % at 100 mg/L and 10% at 500 mg/L. Percent recovery is 104 +/- 7% at 100 mg/L, 99 +/- 7% at 500 mg/L and 100 +/- 7% at 1000 mg/L. Known Gas Chromatography/ Mass Spectrometry (GC/MS) positive GHB urine samples (n=3) were evaluated by this new procedure. Correlation studies of these urine GHB positive samples by this method versus GC/MS gave a slope of 1.32, y-intercept of -587 and correlation coefficient of 1.0. Advantages of this procedure are the small sample volume needed for analysis and the short preparation time, no preparation time is needed, prior to analysis. There are no extraction, derivatization or heating steps required prior to analysis. We conclude that a simple, rapid sensitive and inexpensive method has been presented for the identification and quantitation of GHB in urine. The sensitivity of the method is less than 25 mg/L.

Key words: Gammahydroxybutyric acid (GHB), Analysis, Urine

P6

Carbon Monoxide-Related Deaths in Cuyahoga County, Ohio, 1988 to 1998

Amanda J. Jenkins,[1*] *Cynthia D. Homer,*[2] *David A. Engelhart*[1] *and Eric S. Lavins*[1]. [1]*Cuyahoga County Coroner's Office, Cleveland, Ohio 44106,* [2]*John Carroll University, University Heights, Ohio 44118*

Carbon monoxide (CO) poisoning as a cause of death is well documented in industrialized countries. Regional studies have reported demographic data on these deaths as a result of fires, malfunctioning appliances, or suicidal acts. The objective of this study was to compare demographic data in deaths due to accident (in fires) and suicides in the same population between 1988-1998. Furthermore, the potential effect of a community wide education effort regarding safety in the home was assessed.

Postmortem reports for all deaths examined at the Office of the Cuyahoga County Coroner (CCCO) in Cleveland, Ohio, were reviewed. Cuyahoga County occupies 438 square miles in NE Ohio and includes the city of Cleveland. Data was divided into two groups, namely, accidental deaths by CO resulting from fire in the home and suicides involving CO. Census data, obtained from the US Census Bureau was utilized to compute death rates.

During the eleven year period studied, 32,759 deaths were investigated by CCCO. There were 209 accidental deaths due to fires in the home (6.5% of all deaths attributed to accidents in the home), and 182 CO deaths by suicide (9.8% of all suicides). The number of unintentional (fire) deaths decreased by 80% during the study period. Males represented 55% of the cases, and one half of the victims were black. Race specific death rates were higher for blacks than whites (29/100,000 black, 11/100,000 white). Age specific death rates were highest for the young and elderly. There were 71 deaths in children between the ages of 0-9, and 11 deaths (22/100,00) among those aged over 80. The majority of these deaths occurred in the city of Cleveland. The maximum number of deaths occurred during the month of December, with the minimum occurring in August.

The number of intentional deaths decreased by 41% during the study period. Males represented 70% of the cases, and 94% were white. Race specific death rates were several times higher for whites than blacks (18/100,000 white, 3/100,000 black). Age specific death rates were highest for those over 70 years (25.5/100,000 aged 70-79, and 22.1/100,00 for 80 and older). The majority of these deaths occurred in the suburbs of the county. The maximum number of deaths occurred during the month of April in any year, with the minimum occurring in December.

In October, 1992, the Cleveland Fire Department began a program to distribute smoke detectors to homes in the area. In 1992 there were 4.2/100,000 deaths due to fires in Cleveland. This decreased dramatically to 0.6/100,000 deaths in 1996. This is indirect evidence that the program may have aided in decreasing these types of deaths. However, in 1997 there was an increase in the death rate to 1.2/100,000 followed by a decrease to 0.8/100,000 in 1998. Deaths rates due to fire in the suburbs remained <1/100,000 throughout the study period. Therefore, the long term effects of the program are yet to be determined. Issues of smoke detector maintenance were not addressed. This study demonstrated that deaths due to CO poisoning differ demographically depending upon the manner.

Key words: Carbon monoxide, Fires, Suicide

P7

An Analysis of Drug Findings in Drug Facilitated Sexual Assault Cases

George F. Jackson, *Ph..D. and William A. Dunn, M.S. National Medical Services, Inc., 3701 Welsh Road, Willow Grove, PA 19090*

A retrospective analysis of Drug Facilitated Sexual Assault (DFSA) cases submitted to National Medical Services was conducted. The data represents a tabulation of analytical findings in 399 DFSA cases submitted to National Medical Services from January 2000 through March 2002. Cases were analyzed for demographic information and drug findings.

Urine comprised 70% of specimen types submitted for analysis (blood constituted the remaining 30%). Of all the specimens submitted, 35.6% were negative for drugs. Negative findings in blood and urine were 48% and 31% respectively. None of the cases contained incident time information. Only 72% of cases contained age and sex information. Of those cases, 92.6% involved females and 7.4% males. The mean age for those cases containing demographic information was 21.5 years with a standard error of the mean of 0.5 years and mode of 17 years. The age range was 2 to 57 years. The median age for this group was 19 years. Each DFSA submission to the laboratory was subject to differential extraction and analysis by immunoassay, gas chromatography, liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry for therapeutic and illicit drugs.

The drug found to be in the highest frequency in DFSA positive cases was ethanol (26%), followed by decongestants/antihistamines (17%) and cannabinoids (16%). 64% of all cases were positive with either ethanol alone or in combination with other potentially impairing agents. Further analysis of positive cases indicated that 9% of the cases contained amphetamines followed by narcotic analgesics (8%), benzodiazepines (8%), SSRI's (6%), GHB (4%), barbiturates and related compounds (3%) and cocaine/cocaine metabolites (3%). Analysis of the Decongestants/Antihistamine group for all DFSA positive case showed that ephedrine (30%) and pseudoephedrine (29%) had the highest incidence followed by phenylpropanolamine (17%). In the amphetamine group, amphetamine had the highest incidence (32%) followed by methamphetamine (30%). For the benzodiazepine group, alprazolam and hydroxy-alprazolam (34%) had the highest incidence. Fluoxetine and norfluoxetine (38%) had the highest incidence in the SSRI category. Morphine and hydrocodone had an incidence rate of 22% and 14% in narcotic analgesics respectively.

Key words: Drug-facilitated sexual assault, Drugs, Statistics

P8

GC/MS Method for the Detection of Citalopram in Biological Matrices

David L. Burrows*[1], *M.S.; Andrea N. Hagardorn*[1], *B.S.; Gretel C. Harlan*[2], *M.D.; Ellen D.B. Wallen*[2], *M.D.; Kenneth E. Ferslew*[1], *Ph.D.* [1]*Section of Toxicology, Dept. of Pharmacology;* [2]*Dept. of Forensic Pathology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614*

A gas chromatographic/mass spectrometric assay for citalopram (Celexa®) (a selective serotonin reuptake inhibitor) has been developed for the purposes of either confirming primary drug screen results or quantitation of citalopram in forensic specimens. Plasma and urine matrices were alkalinized (2M NaOH, pH >9.2) and spiked with clomipramine as an internal standard, then extracted into a hexane:isoamyl alcohol solution (99:1) which was evaporated and the residue reconstituted in 50 L methanol. A 4.0 L aliquot of the extracted samples was subjected to the following gas chromatographic/mass spectrometric conditions: injection temperature 250^{o}C, on a 30m x 0.25mm DB-5 capillary heated from 200^{o}C to 270^{o}C at 10^{o}C/min, and subsequent single ion monitoring of masses 324 and 314 for citalopram and clomipramine, respectively.

Analyses were performed on five intraday and five interday specimens consisting of 0.25 ug/mL and 7.0 ug/mL spiked urine and plasma samples to validate our method. The intraday assays yielded a of C.V. <11.15%, and the interday assays yielded a C.V. of <5.51%. All samples were quantitated against a four point standard curve with a mean correlation coefficient (r^2) ≥0.9961. Two forensic cases involving a variety of drugs were analyzed by our newly developed method to show applicability to postmortem specimens. Toxic and lethal plasma concentrations of citalopram, 0.21 and 1.72 ug/mL, were detected and correlated with urine citalopram concentrations of 15.1 and 21.4 ug/mL, respectively. Citalopram analysis on either matrix was not compromised by the presence of multiple drugs, including other tricyclic antidepressants. Method validation and forensic specimen results demonstrate the applicability of our method for determination of citalopram concentrations in biological specimens.

Key words: Citalopram, GC/MS, Quantitation

P9

A Fatal Drug Interaction Between Oxycodone and Clonazepam

David L. Burrows*[1], *M.S.; Andrea N. Hagardorn*[1], *B.S.; Gretel C. Harlan*[2], *M.D.; Ellen D.B. Wallen*[2], *M.D.; Kenneth E. Ferslew*[1], *Ph.D.* [1]*Section of Toxicology, Dept. of Pharmacology;* [2]*Dept. of Forensic Pathology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614*

A case is presented of a fatal drug interaction caused by ingestion of oxycodone (Oxycontin®) and clonazepam (Klonapin®). Oxycodone is an opium alkaloid used in long term pain management therapy. Clonazepam is a benzodiazepine used for the treatment of seizures and panic disorders. The Drug Abuse Warning Network (DAWN) has reported an increase of 108% in the last two years of emergency department episodes related to Oxycontin®. Six billion prescriptions were written for Oxycontin® in the year 2000, an 18-fold increase from four years previous. Oxycontin has recently gained enormous notoriety at the local and national level, however there are very few previously documented cases of lethal drug interactions between oxycodone and clonazepam. Synergistic effects between these two drugs are postulated to arise from different agonistic mechanisms producing similar physiological changes. It is also theorized that clonazepam may inhibit the metabolism of oxycodone. A thirty eight year old white female was found dead in Jefferson County, Tennessee in March of 2001. The deceased had physical evidence of previous drug abuse and positive serological findings of hepatitis B and C. Prescription pill bottles filled under the name of the deceased, as well as another name, were found with the body. Blood, urine and gastric contents from the deceased were screened for numerous drugs and metabolites using a combination of thin layer chromatography and immunoassay techniques (EMIT and FPIA).

Analysis of biological specimens from the deceased revealed the presence of benzodiazepines, opiates (oxycodone), and trazodone metabolites in the blood; cannabinoids, benzodiazepines, opiates (oxycodone), trazodone, trazodone metabolites, nicotine, and nicotine metabolite in the urine; and benzodiazepines, opiates (oxycodone), nicotine, and nicotine metabolite in the gastric contents. Quantitative analyses for clonazepam was performed by high performance liquid chromatography (HPLC) and revealed a blood concentration of 1.41 μg/mL. Plasma oxycodone and urine 11-nor-carboxy-delta-9-tetrahydrocannabinol concentrations were determined by gas chromatography/mass spectrometry and revealed concentrations of 0.60 μg/mL and 27.9 ng/mL, respectively. The deceased had pathologies consistent with severe central nervous system (CNS) and respiratory depression produced by high concentrations of clonazepam and oxycodone including collapsed lungs, aspirated mucus, and heart failure. The pathologies were sufficient to cause death, which was officially attributed to a drug overdose, however the manner of death was unknown.

Key words: Oxycodone, Clonazepam, Drug interaction

P10

Postmortem Blood Ketamine Distribution in Two Fatalities

Brendon R. Lalonde* *and H. Rachelle Wallage. Centre of Forensic Sciences, Toronto, Ontario, Canada*

Despite the reported increased use of ketamine as a recreational drug, relatively few fatalities attributed to ketamine poisoning have been documented. Two recent fatalities in which ketamine was detected are described and compared with cases previously reported in the scientific literature. In Case #1, a 26 year-old man was found in a kneeling position with a rubber tourniquet tied around his arm. A syringe with a needle, along with two 10 cc vials of Ketalar® (ketamine HCl, 50 mg/mL), one empty and one half-full, were found nearby. The deceased was a respiratory technician at a hospital. Ketamine would have been stored for use in this hospital for the treatment of status asthmaticus or for use as an anesthetic, and as such may have been available to the deceased. The death was concluded to be accidental, and the cause of death was ruled to be ketamine intoxication. In Case # 2, a 20 year-old man was at an "all-night party" and after returning home became short of breath and began wheezing. He was taken to the hospital and subsequently died. The administration of ketamine in the emergency room for the treatment of status asthmaticus cannot be ruled out, as the provided history did not include any information regarding medical treatment. Postmortem examination revealed stigmata of asthma; the cause of death was ruled to be asthma.

A general drug screening procedure capable of detecting chemically basic drugs, including ketamine, using gas-chromatography with nitrogen-phosphorus detection (GC/NPD), and confirmation by gas-chromatography-mass spectrometry (GC-MS), was used to screen the blood samples in each case. For quantification in blood, ketamine was extracted using a modification of the basic extraction procedure used for general drug screening. Briefly, toluene and ammonium hydroxide were added to 2 mL of sample blood. The resultant organic layer was separated and sulfuric acid (2N) was added. The aqueous layer was separated and made alkaline by drop-wise addition of sodium hydroxide (5N) and the toluene extract, containing ketamine, was removed. Ketamine was measured in the heart and femoral blood samples using GC/NPD equipped with a DB-1 megabore column (Agilent Technologies), maintained at a temperature of 260°C and a FFAP widebore column (Agilent Technologies), maintained at a temperature of 170°C.

The ketamine concentrations detected in Case #1 were 6.9 and 1.8 mg/L in heart and femoral blood, respectively. In this case, the ketamine concentration detected in the heart blood is in agreement with the lowest concentration reported in the literature, in which the cause of death was ruled to be monointoxication with ketamine. The ketamine concentrations detected in Case #2 of 1.6 and 0.6 mg/L in heart and femoral blood, respectively, were judged to be incidental. Marked differences between heart and femoral blood ketamine concentrations were observed in both of the reported cases. This may be indicative of incomplete distribution prior to death and/ or postmortem redistribution of ketamine.

Key words: Ketamine, Postmortem redistribution, Overdose

P11

Simultaneous Quantification of Psychotherapeutic Drugs in Human Plasma and Whole Blood by Tandem Mass Spectrometry

Wood M* *and Morris M. Clinical Applications Group, Micromass UK Limited, Floats Rd, Wythenshawe, Manchester, M23 9LZ, UK.*

Recent figures indicate that approximately a quarter of the world's population will suffer from a diagnosable mental disorder at some point in their lives. Depression, schizophrenia, anxiety and substance abuse are amongst the most common conditions in the developed countries. Treatment usually comprises counselling and psychotherapeutic medication. For the latter, therapeutic drug monitoring (TDM) is advocated as a means to establish safe, target therapeutic concentrations and also to verify compliance. Amongst the techniques often used for this purpose *i.e.* immunoassay and chromatography, the utility of these methods is often limited due to cross-reactivity and/or insufficient sensitivity. Thus, there is a need for an analytical technique that is both sensitive and specific.

We have developed a simple and rapid LC-MS/MS method that allows the simultaneous quantification of several of the most commonly prescribed psychotherapeutic agents in a single analysis. Drugs were isolated from 50 uL of human plasma using a simple protein precipitation step and subsequently analysed by LC-MS/MS. Chromatographic separation was achieved using a Waters Symmetry 300^{TM} column (2.1 x 150 mm, maintained at 30^{o}C) eluted isocratically with a mixture of ammonium acetate/acetonitrile and delivered at a flow rate of 0.35 mL/min. MS/MS analysis was performed using a Quattro *micro* triple quadrupole mass spectrometer (Micromass UK Ltd) operated in multiple reaction monitoring (MRM) mode. The developed procedure has a total analysis time of less than 20 minutes (including sample preparation).

Calibrators were prepared by adding various psychotherapeutic drugs (including; tricyclic antidepressants, selective serotonin reuptake inhibitors and antipsychotics) to blank plasma. Linear responses were obtained for all analytes (r^2 >0.99) over the range investigated (0.1-500 ug/L). Limits of detection of 1 ug/L (or better) were achieved, which is in accordance with the required sensitivity for TDM of these compounds. The precision of the assay was evaluated by performing replicate (n=6) extractions of plasma samples containing low, medium and high concentrations of the psychotherapeutic drugs (*i.e.* 2, 50 and 200 ug/L, respectively). Coefficients of variation (% CV's) were <20% in all cases.

In order to validate the developed method, plasma samples (n=30), collected from patients currently receiving psychotherapeutic medication, were analyzed by LC-MS/MS and the results compared to those obtained using the current HPLC method. Good correlation (r^2 values >0.9) was obtained between the two techniques.

The LC-MS/MS method has also been used for the analysis of human whole blood and mouse plasma.

Key words: Psychotherapeutics, LC-MS/MS

P12

Drug-Impaired Driving in New Mexico: A Six-Year Retrospective Study

Steven Martinez*, Philip Enriquez and Sarah Kerrigan. New Mexico Department of Health, Scientific Laboratory Division, Toxicology Bureau, Albuquerque, NM, U.S.A.*

Objective: To understand trends in drug-impaired driving among fatally injured New Mexican drivers between 1995 and 2000.

Methods: Drug impaired driving is under-reported, often goes unrecognized and is more difficult to prosecute than alcohol impaired driving. Although the full impact of drugs on traffic safety is relatively unknown, the Department of Health and Human Services report that as many as 9 million people use drugs prior to driving. Drugs other than alcohol were detected in 17.8% of fatally injured drivers nationwide and the prevalence of drugs in surviving drivers involved in crashes has been shown to be even higher. The prevalence of drugs in fatally injured New Mexico drivers was investigated over a six-year period (1995-2000). Blood and urine samples collected at autopsy were tested for nine common classes of drugs using immunoassay, thin layer chromatography and gas chromatography-mass spectrometry. A Microsoft Access database was also developed to collect data from drivers arrested for driving while intoxicated (DWI). The database was used to gather pertinent information such as reason for the stop, driver demographics, the time of day, signs of impairment and driving behavior.

Results: Drugs other than alcohol were detected in 22% of fatally injured drivers. Alcohol (>0.08 g/dL) was detected in 43%, with a median and mode blood alcohol concentration in excess of 0.2 g/dL. Cocaine (13%), cannabinoids (11%), benzodiazepines (5%) and amphetamines (5%) were the most prevalent drugs. The total number of drug mentions was 54%, exceeding that of alcohol (43%). The total drug mentions exceeds the overall positive drug rate of 22% due to multiple drug use in many of the cases. National trends indicate that young people are more likely to drive after drug use. Epidemiological data in New Mexico confirms this trend. Almost half of drug-impaired drivers arrested for DWI were 30 years of age or less. Less than 25% were over the age of forty.

Conclusion: The incidence of drugs in fatally injured New Mexico drivers exceeds estimates from the National Highway and Traffic Safety Administration. This retrospective study highlights the need for action surrounding the role of drugs on driving.

Keywords: Drugs, Driving, Impairment

P13

Validation of Automated Microplate Immunoassays for Eight Drugs-of-Abuse Using Postmortem and Antemortem Fluids and Tissues

Donna Honey*, *Ginger Salazar, Susie Mazarr-Proo and Sarah Kerrigan. New Mexico Department of Health, Scientific Laboratory Division, Toxicology Bureau, Albuquerque, NM, U.S.A.*

Objective: To evaluate eight automated enzyme-linked immunosorbent assays (ELISAs) in terms of analytical performance and casework reliability.

Methods: Commercial immunoassays for barbiturates, benzodiazepines, cannabinoids, cocaine metabolite, methadone, methamphetamine, opiates and propoxyphene, were evaluated using a fully automated analyzer. The analytical performance of each assay was evaluated using the following criteria: limit of detection, dose-response curve, EC50, precision, matrix effects and binding characteristics at the cutoff concentration. Target drugs for each of the assays were secobarbital, nordiazepam, *l*-11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCA), benzoylecgonine (BE), methadone, *d*-methamphetamine, morphine and propoxyphene. The reliability of each assay was determined using case samples consisting of antemortem blood and urine and postmortem blood, urine, liver and brain. More than two hundred case samples of known composition by GC/MS were screened using five potential cutoff concentrations. Sensitivity, specificity, positive and negative predictive values were calculated at each cutoff concentration. Receiver operating characteristic (ROC) analysis was used to determine the appropriate cutoff concentrations.

Results: Limits of detection was variable and ranged from less than 1 ng/mL to 42 ng/mL. Precision was estimated using negative controls and calibrators prepared in-house. Mean covariance (CV) in drug-free blood and urine was 2.4–4.9 and 3.3–10.2% respectively. Dose-response curves in blood and urine showed some differences between matrices. The propoxyphene, cannabinoid, benzodiazepine and methamphetamine assays were most susceptible to matrix effects. These were mitigated by running blood and/or urine calibrators and controls. EC50 values in whole blood were 12, 32, 13, 180, 29, 83, 65 and 67 ng/mL for secobarbital, nordiazepam, THCA, BE, methadone, methamphetamine, morphine and propoxyphene. Cutoff concentrations were selected to optimize assay reliability. Whole blood cutoffs were 100 ng/mL for all assays except cannabinoids (50 ng/mL). Sensitivity and specificity ranged between 72-100% and 86-100% respectively (n=110). Urine cutoffs were 500 ng/mL for BE, methamphetamine, propoxyphene and opiates, 200 ng/mL for methadone, benzodiazepines and barbiturates and 100 ng/mL for cannabinoids. Sensitivity and specificity were 88-100% and 49-100% respectively (n=68).

Conclusion: Selection of the appropriate cutoff concentration allowed presumptive screening of postmortem and antemortem fluids and tissues using automated ELISAs. Assays were rapid, reliable and required no pretreatment other than dilution with buffer solution.

Key words: Validation, Immunoassay, Drugs of abuse

P14

Validation of the Cozart Microplate ELISA for Detection of Opiates in Hair

***Gail Cooper*[1]*, *Lisa Wilson*[1], *Claire Reid*[1], *Dene Baldwin*[1], *Chris Hand*[1], *and Vina Spiehler*[2].
[1]*Cozart Bioscience Ltd, Abingdon, Oxfordshire OX14 4RU, UK;* [2]*Spiehler and Associates, Newport Beach, CA, 92663*

The purpose of this study was to determine the performance characteristics of the Cozart Opiates Microplate ELISA assay for the detection of opiates in hair specimens. 92 hair specimens were collected from volunteers in Drug Clinics and from drug-related deaths. The samples were extracted by sanitation of approximately 20-30 mg of hair in 2 mL of methanol for 60 min followed by extraction overnight at 60°C. The methanol extract was then blown to dryness and reconstituted in 500 µL Negative Calibrator matrix. 25 µL of the reconstituted extract was added to each well of the coated microtiter plate followed by 100 µl of working enzyme conjugate. Samples were run in duplicate as recommended by the manufacturer. After a 30 min incubation, the plate was washed four times with 350 µL wash buffer. Then 100 µL of substrate solution containing 3,3',5,5'-tetramethyl benzidine as the chromogen (TMB) was added to each well and incubated for a further 30 min. Finally 100 µl of stop solution (1 M sulphuric acid) was added to each well and the absorbance was read at 450 nm using a microplate reader within 30 min. Concentrations were determined from the assay calibration curve run on the same plate as the reconstituted specimen extracts. Calibration standards were made up in blank hair spiked with morphine to concentrations of 0, 50, 100, 200, 300, 500 and 1000 pg/mg. The precision of the Cozart Opiates Microplate ELISA at 200 pg/mg morphine equivalents in hair was: intraassay, mean absorbance 0.48 ± 0.02 SD (4.5 %CV), n = 20 and interassay, mean absorbance 0.59 ±0.04 SD (5.99 %CV), n = 5. The cross-reactivities of the ELISA determined from buffer spiked with drug at 5 ng/ml were codeine 500%, pholcodeine 240%, dihydrocodeine (DHC)100%, and morphine 100%; and, spiked at 100 ng/ml, heroin 17%, and 6-acetylmorphine (6-AM) 14%.

For GC/MS analysis, 50 µL of deuterated internal standard mixture and 1 ml 0.1 M HCl was added to 20 mg of specimen or spiked blank hair (0, 0.2, 0.5, 1.0, 2.5, or 5.0 ng/mg DHC, codeine, morphine, 6-AM and heroin) and sonicated for 1 hour. The opiates were extracted by solid-phase (BondElut Certify 50 mg, 3 ml) and derivatized with 100 µl BSTFA + 1% TMS at 70°C for 20 min. The GC/MS was an Agilent 5973N + 6890 GC-EI with splitless injection equipped with J&W DB-5MS, 30 m, 0.25 mm, 0.25 µm. capillary column, run at 150°C (0.5 min) ramped at 17.5°C/min to 290°C (6 min). With a 20 mg hair sample, the LOD/LOQ was 0.1 ng/mg hair for all the opiates. 40 hair specimens were confirmed positive by GC/MS. Opiates found by GC/MS in the hair specimens included morphine (26 specimens), DHC (11 specimens) codeine (17 specimens), 6-AM (26 specimens) and heroin (7 specimens). Opiate hair concentrations ranged from 0.26-4.4 ng/mg morphine, 0.13-7.28 ng/mg 6-AM, 0.28-1.66 ng/mg heroin, 0.12-2.19 ng/mg codeine and 0.32-23.3 ng/mg DHC by GC/MS.

(continued)

The true positives, true negatives, false positives and false negatives for different cutoffs with the ELISA were determined by comparison of each individual ELISA response (normalized to weight of hair extracted) to the GC/MS results as the referee method. Sensitivity was calculated as: TP/(TP + FN); Specificity as: TN/(TN + FP. The optimum cutoff was determined by plotting Sensitivity vs (1-Specificity) (ROC plot). From ROC analysis the optimum cutoff for the Cozart Opiate Microplate ELISA was determined to be 200 pg morphine equivalents/mg hair. The Cozart Opiates Microplate EIA for opiates in hair using a cutoff of 200 pg/mg hair had a sensitivity of 97.5% ± 2.5% and a specificity of 92.3% ± 3.7 % vs GC/MS. There were four false positives which may have been due to cross-reactivity with pholcodeine which is not detected by the GC/MS assay and one false negative at a cutoff of 200 pg/mg hair.

Key words: Opiates, Hair, ELISA

P15

Evaluation of the Triage PPY On-site Testing Device for Detection of Dextropropoxyphene in Urine

*Alphonse Poklis[1], **Justin L. Poklis***[2], Lisa Tarnai[3] and Ronald C. Backer[4]. [1]Departments of Pathology and [2]Pharmaceutical Sciences, Medical College of Virginia Campus at Virginia Commonwealth University, Richmond, VA 23298-0165; [3]Scientific Testing Laboratories, Inc., Richmond, VA; [4]Ameritox, Midland, TX*

We evaluated the clinical performance of a new colloidal metal immunoassay device [TRIAGE® Plus propoxyphene (TPP)] which was designed for the rapid detection of dextropropoxyphene (PPY) and/or its primary metabolite, norpropoxyphene (NP) in urine at concentrations of 300 and 450 ng/mL or greater, respectively. This assay has been added to a device that also detects the presence of tricyclic antidepressants and seven commonly abused drugs. The linearity and selectivity of the TPP assay was determined by testing gravimetrically prepared urine samples. TPP yielded a positive response to PPY at the stated cut-off value (300 ng/mL) and 120% of cut-off (360 ng/mL). Samples containing PPY at 80% of cut-off (240 ng/mL) were negative. Similarly, samples containing NP at the stated cut-off value (450 ng/mL) and 540 ng/mL tested positive, while those at 360 ng/mL were negative. No significant cross reactivity was found for 32 drugs commonly encountered in emergency department admissions tested at 100 mg/L. TPP results from testing 150 probation/parole and pre-employment urine specimens for PPY and/or NR were compared to those obtained by DRI enzyme immunoassay (DRI), Emit II plus immunoassay (Emit), Abuscreen Online immunoassay (Online) and gas chromatography/mass spectrometry (GC/MS). Briefly, GC/MS analysis for PPY and NP involved the liquid/liquid extraction and base hydrolysis of NP to the cyclic amide derivative. Identification and quantitative analysis was by SIMS monitoring of the following ions (m/z): 58,208,115 (PPY); 234,100,91 (NP cyclic amide) and 86,99 (SKF-525, internal standard). The LOQ and LOD of the GC/MS assay for both PPY and NP were 50 and 100 ng/mL, respectively.

There was complete agreement of findings between TPP, DRI and OnLine, 100 positive results and 50 negative findings. A 98% (98/100) agreement of positive results was found between TPP and GC/MS. The two discordant results were due to NP concentrations within 20% of the cut-off value of the TPP assay. There was only an 87% agreement of positive or negative results between TPP and the Emit assay. Twenty urine specimens yielding PPY positive results by TPP were negative by Emit testing. These discordant TPP results were due to poor cross-reactivity of Emit to NP. This study found TPP to be an accurate and selective device for the detection of PPY and/or NP in urine.

Key words: Triage, On-site testing, Propoxyphene, Urine drug testing

P16

An Accurate Method for the Determination of Carboxyhemoglobin in Postmortem Blood using GC/TCD

Russell J. Lewis*, Robert D. Johnson, Dennis V. Canfield. Federal Aviation Administration, Civil Aerospace Medical Institute, Forensic Toxicology, Oklahoma City, Oklahoma, U.S.A.*

During the investigation of aviation accidents, postmortem samples from accident victims are submitted to the FAA's Civil Aerospace Medical Institute for toxicological analysis. In order to determine if the accident victim was exposed to an in flight/post crash fire, or faulty heating/exhaust system, the analysis of CO via carboxyhemoglobin (COHb), is conducted. While our laboratory predominantly uses a spectrophotometric method for the determination of %COHb, we consider it essential to confirm with a second technique based on a different analytical principle. Our laboratory encountered difficulties with many of our postmortem samples while employing a commonly used GC method. Therefore, an alternative GC method was sought.

It is well known that methemoglobin (MetHb) concentration can be elevated in postmortem blood. Therefore, a reducing agent is commonly employed in the spectrophotometric analysis of COHb to reduce any MetHb in addition to oxyhemoglobin present in the blood. This eliminates their potential interference with the absorbance of COHb. To the best of our knowledge no one has yet reported the use of a reducing agent in conjunction with the GC determination of %COHb. This reduction is extremely important in a postmortem specimen, which has the potential to contain high levels of MetHb. This importance stems from the fact that the commonly employed GC methods determine %COHb from a ratio of unsaturated blood to CO-saturated blood. Since MetHb does not bind CO, elevated MetHb levels will result in a loss of CO binding capacity, resulting in a less than complete hemoglobin CO saturation, thus producing an erroneously high %COHb value.

Our laboratory has developed a GC-CO method for the determination of %COHb that incorporates sodium dithionite as a reducing agent. Using numerous fresh human blood controls ranging from 1% to 66% COHb, we found no statistically significant differences between %COHb results from the GC-CO method and our spectrophotometric method. We then employed the new GC-CO method to putrefied and non-putrefied postmortem samples. For comparison the postmortem samples were analyzed with our existing spectrophotometric method, a GC method commonly used without reducing agent, and the same GC method with the addition of sodium dithionite. As expected, we saw errors up to and exceeding 50% when comparing the unreduced GC and spectrophotometric methods. We found the error in the unreduced method was directly attributable to the amount of MetHb present in a sample. When our new GC-CO procedure was employed the error was virtually eliminated.

The presence of MetHb in specimens can spuriously elevate CO results using GC methods previously published in the literature. GC/TCD can be an accurate and reliable method for the determination of COHb in postmortem blood if proper precautions are taken to reduce any MetHb in the sample.

Key words: Carbon monoxide, Carboxyhemoglobin, Methemoglobin

P17

Adulterants: Its Detection and Effects on Urine Drug Screens

Baguio Wong*[1], *Phuong Nguyen*[1], *Raphael Wong*[1] *and Harley Tse*[2]. [1]*Branan Medical Corporation, Irvine, CA, USA,* [2]*Wayne State University, Detroit, MI, U.S.A.*

The use of commercial adulterants to defeat urine drug tests has become a growing problem for the drug testing industry. Awareness of what these adulterants can do and the availability of the means to detect them are of great importance to forensic toxicologists.

We have established a simple protocol to evaluate the effectiveness of adulterants on POC urine drug screens as follows: Urine drug controls containing three times the SAMHSA cut-off concentrations of morphine (Mor), THC, benzoylecgonine, amphetamine, and PCP were mixed with commercial adulterants according to their respective directions and the presence of the abused drugs in these controls were tested over 24 hour period using an on-site drug screens, Monitect® PC-11 cassettes. Five commercial adulterants were evaluated. After 24 hours, the control samples were sent to a laboratory and tested for the presence of the affected drugs by GC/MS. We also evaluate the use of an on-site adulterant test strip, Intect®7 to detect the presence of these adulterants at zero time. The results are as follows:

	-ve Drug Test Result on Monitect®			-ve GC/MS	Detected by Intect®7
	5 min.	5 hours	24 hours		
Stealth	THC, Mor	THC, Mor	THC, Mor	THC, Mor	Yes
Urine Luck v.6.3	THC	THC, Mor	THC, Mor	THC, Mor	Yes
Purafyzit	None	None	None	None	Yes
Clean-X	None	None	None	None	Yes
InstantClean	THC, Mor	THC, Mor	THC, Mor	THC, Mor	Yes

The results show that some of these adulterants are effective in modifying the drug test results. However, an on-site adulterant test strip is useful to detect their presence in the urine samples.

Key words: Adulterant, Intect 7, Urine drug testing

P18

Development of a Rapid and Sensitive Method for the Quantification of Benzodiazepines in Plasma and Larvae by LC-MS/MS

Michelle Wood*[1], *Gert De Boeck*[2], *Nele Samyn*[2], *Karen Pien*[3], *Patrick Grootaert*[4], *Michael Morris*[1]. [1]*Micromass UK Limited, Wythenshawe, Manchester, UK;* [2]*National Institute of Criminalistics and Criminology (NICC), Section Toxicology, Brussels, Belgium;* [3]*Department of Anatomo-Pathology, Academic Hospital, Free University of Brussels, Belgium;* [4]*Royal Belgian Institute of Natural Sciences, Department of Entomology, Brussels, Belgium*

LC-MS/MS is emerging as the tool of choice for rapid analysis and detection of biologically active compounds in complex mixtures. Here we describe the development of a rapid and sensitive method for the simultaneous quantitation of a panel of 10 benzodiazepines (BDPs). They were isolated from human plasma using a simple acetonitrile precipitation step and subsequently analysed using reversed-phase HPLC-MS/MS. LC was performed using a Waters Alliance 2690 system. Chromatography was achieved using a Zorbax SB-phenyl column (2.1 x 150mm, 5µm) with gradient elution with an acetonitrile, methanol and ammonium acetate mixture delivered at a flow rate of 0.25mL/min. A Quattro *Ultima* triple quadrupole mass spectrometer (Micromass UK Ltd.) fitted with "Z"-Spray ion interface was used for all analyses. Ionisation was achieved using electrospray in the positive ionisation mode (ES+).

Quantification of the BDPs was performed using multiple reaction monitoring (MRM) and integration of the area under the specific MRM chromatograms. In all cases the BDPs were quantified by reference to the integrated area of their respective deuterated analogues. The developed method, which requires only 25µL of biological sample, has a total analysis time of less than 30 minutes (including sample preparation) and enables the simultaneous quantification of alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nordiazepam, oxazepam, prazepam, temazepam and triazolam in a single chromatographic run. Linear responses in plasma were obtained for all the BDPs ($r^2 > 0.99$) over the range investigated (1-800µg/L). For all compounds LOD's of 1µg/L (or better) and LOQ's of 0.2µg/L (or better) were achieved. The precision of the assay was assessed by performing replicate (n = 5) extractions of plasma samples containing low, medium and high concentrations of the BDPs (i.e. 2, 40 and 200µg/L respectively). Coefficients of variation (%CV's) were found to be highly satisfactory (<15%).

The same LC-MS/MS method was used to analyse fly larvae. In addition to their use in the estimation of postmortem intervals, insects may serve as reliable alternate specimens for toxicological analyses in the absence of tissues and fluids normally taken for such purposes. Larvae of the Calliphora vicina fly were reared on artificial foodstuff (beef heart) spiked with a range of concentrations of nordiazepam (0.5µg/g; 1µg/g and 2µg/g). The LOD and LOQ's obtained from spiked larvae were in the same range as those obtained with the plasma samples. The larvae were harvested at day 7 for analysis of drug content. Positive drug concentrations of nordiazepam and its metabolite oxazepam were found after the analyses of one larva.

Further investigations are required to determine the metabolism of nordiazepam in the Calliphora vicina larvae and pupae and the role of the toxic compounds on their development.

Key words: Benzodiazepines, LC-MS/MS, Entomotoxicology

P19

An ONLINE DAT II Immunoassay for the Detection of Methadone in Urine

Daniel K. Hoch, Judy Zhao, Kelley Kreke, Christopher West, Mitali Goshal,Richard Root, Gerald Sigler, Matthew Slagel, Steven Vitone, ***Davina C. Ananias****, and Joseph Passarelli. Roche Diagnostics Corporation, 9115 Hague Rd., Indianapolis, IN 46250*

An ONLINE Generation II immunoassay has been developed for the determination of methadone in urine. This two-reagent system utilizes a methadone-specific monoclonal antibody in a reaction that kinetically monitors the inhibition of agglutination of antibody-coated microparticles with drug-polymer conjugate. The new assay offers a broad dynamic range of 0-2000 ng/mL with a 300 ng/mL cutoff concentration. Both semi-quantitative and qualitative applications of the assay are examined on the Roche/Hitachi 917 and the COBAS INTEGRA® 700 clinical analyzers.

Studies demonstrate that the new immunoassay displays a low background, with a clinical sensitivity (mean + 2 S.D.) of less than 10.1 ng/mL on the Hitachi 917 and 32.4 ng/mL on the Integra 700. A method comparison of 100 clinical samples that were screened negative by the EMIT® immunoassay gives negative sample results with the ONLINE Methadone II assay; and 55 GC/MS confirmed clinically positive samples result in positive values with the new assay system. Testing on ten samples that challenge the 300 ng/mL cutoff (225-241 ng/mL and 310-375 ng/mL) provides correct qualitative responses with the assay. Further studies show good precision and recovery of control samples across the range of the curve. The median semi-quantitative intra-assay CV% of the 150, 225, 300, 375, and 1500 ng/mL controls (n=20) for the Roche/Hitachi 917 are 1.9%, 2.1%, 1.3%, 1.4%, and 3.4%, respectively; and the respective CV% values for the COBAS INTEGRA® 700 are 3.8%, 2.4%, 2.0%, 2.2%, and 3.7%. Inter-assay precision of the same levels (n=20 x 5 calibrations) give CV% values of 2.3%, 2.8%, 2.7%, 2.5%, and 3.5% on the Hitachi 917 and 4.4%, 3.5%, 2.5%, 2.2%, and 5.2% on the Integra 700. The assay is specific for methadone with no cross–reactivity to EDDP or EMDP and 0.1% cross-reactivity to LAAM. Studies also demonstrate extended reagent stability and higher throughput in comparison to the OnLine Gen I Methadone assay and come ready to use with no reagent reconstitution. A shelf life of >18 months is demonstrated using an accelerated model.

Key words: Methadone, ONLINE, Immunoassay

P20

A Quick Simultaneous Method of Detection and Quantitation of Volatile Alcohols and Ethylene Glycol by On Column Capillary Gas Chromatography in Biological Samples

Jayant A. Patel*, *Thomas Annesley, Donald Giacherio. The University of Michigan, Dept. of Pathology, Ann Arbor, MI 48109*

The detection of an unknown volatile substance ingested by patients brought to the Hospital Emergency services require immediate care. The identification of the volatile substances is essential qualitatively and quantitatively within a short time to treat the patient. The nature of volatile substances like alcohols and Glycols requires two separate Gas chromatographic procedures. We are presenting a quick procedure by modifying previously published procedure by Williams(1) et al. A Hewlett Packard Model 6890 equipped with flame ionization detector and Rtx®-200 capillary column at 70°C. was used. A five-point calibration ranging from 12.5 mg/dL to 400 mg/dL was used to determine linearity. The controls used were 50 and 200 mg/dL in water.

The modified sample processing involves simple mixing of the 50 μL of biological specimen (serum, plasma or urine) and 500 μL of acetonitrile containing two internal standards n-propanol and 2-3 butane diol in a micro centrifuge tube. The mixture is vortexed and centrifuged for 0.5 minute and one μL of the supernatant aqueous sample is injected in to a gas chromatograph. The results of Methanol, Ethanol, Isopropanol, acetone and ethylene glycol are available within 15 minutes. The patient results determined by alternate gas chromatographic method were comparable. There was no carryover of analytes determined after highest concentration and running a blank water injection. The LOD 5 mg/dL and LOQ was 10 mg/dL. for each analyte.

Analysis of a sample spiked with 100 mg/dl of each volatile component over a one month period (n=31) yielded the following data for:

Component	Mean mg/dl	Standard deviation	CV%
Methyl alcohol	97.56	5.23	5.4
Ethyl alcohol	99.33	3.97	4.0
Isopropyl Alcohol	100.22	4.17	4.2
Acetone	98.22	5.74	5.8
Ethylene Glycol	101.43	8.89	8.8

The other volatiles like Diethylene glycol, Propylene glycol and γ- Hydroxy Butyrate(GHB) are also detectable by this method.

Key words: Volatiles, Ethylene glycol, Gas chromatography, Biological specimens

P21

Detection of Club Drugs and Drugs Associated with Drug-Facilitated Sexual Assault in Human Urine by Immunoassay

B.A. Mayer*, T.A. Heller and D.E. Schroedter. Neogen Corporation, Lexington, KY 40505*

In recent years, powerful drugs like methamphetamine and MDMA (Ecstasy) have become popular in the "club" scene because of the stimulant and euphoric affects associated with their use. Other drugs such as ketamine and flunitrazepam (Rohypnol) have become prevalent in drug-facilitated sexual assault (DFSA). To help in the detection of these compounds in such cases, investigators could benefit from rapid and accurate screening methods. Immunoassays have proven invaluable as screening methods in many areas of drug detection because of their ease of use, low cost, and high throughput of samples. The objective of this evaluation is to demonstrate the benefit to the forensic industry of immunoassays for the detection of club/DFSA drugs and metabolites in human urine. The sensitivities in human urine reported below should prove adequate for use when screening for the presence of the "club drugs"/DFSA drugs.

The evaluation of three Neogen competitive immunoassays for use with human urine samples was completed. Neogen's Methamphetamine, Flunitrazepam and Ketamine immunoassays were evaluated for matrix affects using forty-three blank human urine samples. Based upon the screening of these blank human urine samples, it was necessary to dilute samples 1:10 (one part sample with ten parts sample buffer) on the Methamphetamine assay to eliminate matrix interference with human urine. However, only a dilution of 1:3 was required to remove matrix interference in the Flunitrazepam assay. No sample dilution was required in the Ketamine assay.

The sensitivity of each of assay was evaluated by generating standard curves with the drug, analog, or metabolite in blank human urine. These standards were diluted accordingly (see above) with assay buffer and assayed. The sensitivity for these compounds was expressed as an I_{50} (the drug concentration that provides one half maximal binding in the assay) or as a LOD (backfit concentration of zero standard calculated from average OD_{650} +/- 2 SD (n = 8), % CV = SD ÷ average OD_{650}).

The sensitivities for these drugs and/or metabolites in human urine were as follows:

Compound	Urine Dilution (sample: buffer)	I_{50} (ng/mL)	LOD (ng/mL)	% CV
Methamphetamine	1:10	90	4.7	3.6%
p-Hydroxymethamphetamine	1:10	120	1.3	2.3%
MDMA	1:10	57	2.8	2.9%
PMMA	1:10	34	2.0	3.1%
Flunitrazepam	1:3	40	1.2	2.4%
7-Aminoflunitrazepam	1:3	7	0.4	4.5%
Ketamine	No Dilution	10	0.3	3.4%
Norketamine	No Dilution	200	15	4.0%

Key words: Club drugs, Urine, Immunoassay

P22

Determination of Zopiclone Misuse in the Republic of Ireland Using GC-MS

Richard Maguire,[*] *Columba Moran, Dymphna Talbot, Mark Dowling, John O'Connor and Siobhan Rooney. Drug Treatment Centre Board, Trinity Court, 30-31 Pearse Street, Dublin 2, Republic of Ireland*

Although structurally unrelated, zopiclone and the benzodiazepines, share certain pharmacological activity. Zopiclone is a cyclopyrollone, which possesses hypnotic and sedative effects as well being an anticonvulsant and muscle relaxant. Marketed as Zimovane® by Aventis Pharma in the Republic of Ireland for use as a sedative, its misuse has been established outside of Ireland. It was suspected that clients attending Ireland's largest addiction treatment centre were misusing zopiclone. However until recently there has been no method available of detecting the use of zopiclone in our laboratory. This coupled with the drug's similarity of effect to the benzodiazepines has lead to the misuse of zopiclone.

Typical therapeutic concentrations for zopiclone in blood are 18.4-35.2 ng/mL. A post mortem involving an overdose of zopiclone and pentazocine reported levels of zopiclone in the blood to be 1.18 μg/mL. Zopiclone itself is not the most useful target for the detection of use/misuse as it undergoes metabolism to *N*-oxide and *N*-demethylated derivatives. These metabolites are excreted in urine (36% of original dose) along with small amounts of unchanged drug. Galloway *et al.* proposed the use of GC-MS, targeting the metabolite 2-amino-5-chloropyridine (ACP), a further breakdown product of both *N*-oxide and *N*-demethylated derivatives and the parent drug, for the determination of zopiclone in various matrices. Based on this method, a clinic with a cohort of 150 clients was assessed for the use of zopiclone. Urine samples were made alkaline and extracted with ethyl acetate. The ethyl acetate extract was injected directly into a 6890 Agilent GC coupled to a 5973 Agilent MS. Control urine containing ACP at 0.1μg/mL and 1.0 μg/mL was run along with each batch of samples. Samples were reported positive when ACP was detected. Of the 158 clients tested over a two-month period it was established that 24% were using zopiclone based on the detection of ACP. A further experiment was also carried out to determine suitable derivatization procedures, which may improve the chromatography of ACP, using sylilation and acetylation.

Keywords: Zopiclone, 2-amino-5-chloropyridine, GC-MS

P23

Hyaluronidase as a Liquefying Agent for Chemical Analysis of Vitreous Fluid

***Uttam Garg*[*1],** *Randah Althahabi [1], Mick Brod[1], Thomas Young[2] and Chase Blanchard[2]. [1]Section of Pathology and Laboratory Medicine, Children's Mercy Hospital and [2]Jackson County Medical Examiner, Kansas City, MO*

Vitreous humor is a suitable specimen for postmortem clinical chemistry because the analytes remain stable after death and they closely reflect blood levels immediately prior to death. The viscous nature of vitreous fluid, however, presents analytical problems including imprecision, and pipeting errors . Various preanalytic treatments, such as boiling, high speed centrifugation, microfiltration, and dilution have been used, but are labor intensive and add to imprecision.

We used hyaluronidase (HL) as a liquefying agent prior to analyzing aliquots from 24 vitreous specimens with the Vitros Chemistry System (Ortho Diagnostics) and compared the results with pretreatment by dilution alone or no pretreatment. Hyaluronic acid is the predominant glycosaminoglycan in vitreous humor, and the enzymatic removal of hyaluronic acid made the specimens sufficiently fluid for analysis. We needed to dilute half of the non-HL-treated aliquots because of high viscosity. HL treatment (~1 mg/mL) negated the need for sample dilution and had no significant effect on the analyses, as demonstrated in the table below.

(continued)

Table: Effect of HL on chemistry analytes. Blank values (represented by hyphens in the table) indicate either that the analyte could not be measured even after dilution or that the specimen quantity was insufficient. HL treated samples were analyzed without dilution.

		Glucose	**Potassium**	**Sodium**	**Chloride**	**BUN**	**Creatinine**
Sample	**Dilution**	**-HL (+HL)**	**-HL (+HL)**	**-HL (+HL)**	**-HL (+HL)**	**-HL (+HL)**	**-HL (+HL)**
1	None	25(32)	11 (11)	139(141)	125(128)	8(8)	0.4(0.3)
2	1:3	<20(<20)	10(10)	-(148)	-(129)	12(9)	0.9(0.6)
3	1:3	<20(<20)	9(9)	-(141)	-(127)	-(15)	0.4(0.6)
4	None	<20(<20)	-(-)	-(-)	95(100)	14(13)	-(-)
5	None	<20(<20)	-(-)	131(139)	117(122)	7(7)	-(-)
6	None	55(56)	9(10)	134(142)	115(120)	-(18)	1.0(0.8)
7	None	291(304)	-(-)	113(117)	87(90)	53(53)	-(-)
8	None	-(648)	-(8)	-(173)	141(146)	76(67)	1.9(1.4)
9	None	<20(<20)	-(-)	138(141)	123(124)	9(9)	0.8(0.8)
10	1:2	-(-)	9(9)	-(138)	-(120)	31(20)	1.1(1.0)
11	None	<20(<20)	10(11)	135(135)	121(129)	7(7)	0.2(-)
12	1:3	<20(<20)	10(9)	-(142)	-(117)	8(10)	0.4(0.3)
13	1:4	48(37)	8(7)	-(156)	-(138)	6(6)	0.6(0.3)
14	1:4	94(91)	14(14)	-(149)	-(116)	14(14)	1.1(1.3)
15	1:6	173(134)	14(12)	-(142)	-(124)	36(36)	1.2(0.6)
16	1:6	<20(<20)	7(7)	137(135)	119(115)	19(17)	1.1(0.7)
17	1:6	<20(<20)	-(-)	-(137)	130(123)	12(12)	0.9(-)
18	1:6	<20(<20)	12(10)	-(-)	-(-)	11(8)	0.6(0.3)
19	None	<20(<20)	12(12)	130(134)	117(-)	7(-)	0.3(0.3)
20	None	-(-)	9(9)	136(142)	115(119)	-(-)	-(-)
21	None	<20(<20)	9(9)	-(130)	113(117)	20(17)	-(0.9)
22	1:2	<20(<20)	-(7)	-(145)	-(126)	-(17)	-(0.6)
23	1:2	<20(<20)	-(-)	-(129)	-(113)	-(14)	-(0.6)
24	1:2	-(291)	-(9)	-(158)	-(135)	-(28)	-(1.0)

Key Words: Vitreous humor, Hyaluronidase, Postmortem chemistry

P24

Directed Second Sample Analysis of Blood from DUID Arrests in the Commonwealth of Virginia, 1990-1999

Carl E. Wolf* *and Alphonse Poklis. Department of Pathology, Medical College of Virginia Campus at Virginia Commonwealth University, Richmond, VA 23298-0165*

In 1989 the Commonwealth of Virginia began enforcement of new Driving Under the Influence of Drugs statutes that permitted the accused to have a second blood sample drawn at the time the evidentiary sample was collected. The defendant could choose to have this second sample sent to an independent laboratory accredited by the Division of Forensic Sciences. Once the state completed testing the evidentiary sample, the results were sent to the court and a qualitative report was sent to the Second Sample Laboratory (SSL). The SSL then performed quantitative confirmation testing for the drugs identified by the state. The results of SSL testing were sent to the court to be introduced only by the defense if warranted.

I. Demographic and Drug Findings. Demographic and drug findings of SSL testing of (N=2294) blood specimens by year over the ten year period of 1990 to 1999 showed that the commonly encountered drugs as a percentage (mean and range of blood concentrations) over the ten years were: 46% Blood Alcohol (0.06 / 0.01 - 0.42 g/dl, 32% THC/THC-Acid (THC 2 / <1 - 129 ng/ml; THC-Acid 11 / 2 - 215 ng/ml); 20% Cocaine/Benzoylecgonine (Cocaine 20 / <10 - 270 ng/ml; Benzoylecgonine 70 / 8 - 3240 ng/ml); 10 % Diazepam/metabolites (Diazepam 100 / 9 - 2000 ng/ml; Nordiazepam 100 / 9 - 3200 ng/ml); 7% Alprazolam (59 / 7 - 520 ng/ml), 4% Phencyclidine [PCP] (32 / 2 - 101 ng/ml); 3% Carisoprodol/Meprobamate (Carisoprodol 3.5 / 0.6 - 29.0 mg/L; Meprobamate 8.6 / <1 - 61.2 mg/L). Men represented 79% of those requesting second sample testing. Significant trends displayed over the ten year period included an increase in carisoprodol cases and a decrease in PCP cases. The carisoprodol positive cases occurred in western counties of the state and PCP cases were exclusively from the northern counties of the state.

II. Comparison of the Results of Evidentiary Sample and Second Sample Testing. The percentage of agreement of SSL confirmation results with evidentiary sample results for the most commonly encountered drugs over the ten years were: Blood Alcohol 96%, THC 77 %; THC-Acid 93%, Cocaine 64%, Benzoylecgonine 99%; Diazepam 92%, Nordiazepam 97%; Alprazolam , 89%. Phencyclidine [PCP] (100%), Carisoprodol 98%; Meprobamate 98%. Lack of agreement with THC and Cocaine may be due to a delay of 1 - 4 months in receiving the qualitative report of the evidentiary sample. Samples that had insufficient sample volume or a potential interference were excluded.

Key words: Drugs and driving, Drug abuse, Blood analysis

P25

Stability of Olanzapine in Stored Blood

Colleen W. O'Keeffe, William R. Johnson, J. Kim Ricksecker, ***Laura J. Liddicoat****. State Laboratory of Hygiene-Toxicology, University of Wisconsin-Madison U.S.A.*

The antipsychotic agent olanzapine (Zyprexa) is a benzodiazepine analog structurally similar to clozapine. Olanzapine readily oxidizes in preserved blood during storage and extraction resulting in a decrease in the measured drug concentration over time. The addition of ascorbic acid has been shown to stabilize the drug in serum when stored for up to 2 weeks.

Forensic specimens are often stored for extended periods of time prior to analysis. This study investigates the stabilizing effect ascorbic acid exerts on olanzapine in preserved whole blood during storage and the effect ascorbic acid added during extraction has on restoring original olanzapine concentrations.

This study consisted of three phases. First, a stability experiment was set up with blank blood spiked with olanzapine at 100 ng/mL. Blank blood was spiked and then divided into two pools; ascorbic acid (0.05% final concentration) was added to one pool. Next, both pools were aliquotted into 10mL Vacutainer® tubes containing sodium fluoride and potassium oxalate and stored at 4ºC. Samples from each pool were extracted both with and without 0.05% ascorbic acid using a basic n-butylchloride extraction and analyzed by capillary column GC-NPD once a week over a 119 day period. The second phase analyzed forensic specimens extracted with varying concentrations of ascorbic acid (0.03% - 0.20%) and without ascorbic acid using the same n-butylchloride extraction. Finally, the stored olanzapine stability pool was extracted with various concentrations of ascorbic acid (0.05%-0.40%).

For the stability phase, the two storage and two extraction conditions resulted in a total of four distinct conditions. Samples both stored and extracted with ascorbic acid (Group IV) showed the greatest increase in recovery when compared to the control (Group I) in which no ascorbic acid was added at any point. Of all the test conditions, Group IV also showed the greatest stability. When ascorbic acid was added either during storage (Group III) or during extraction (Group II), results demonstrated increased olanzapine recovery but decreased stability.

The remaining two phases compared olanzapine recovery when extracted with various ascorbic acid concentrations versus extraction without ascorbic acid. Six of the eight forensic specimens attained higher olanzapine recoveries whenever any amount of ascorbic acid was added. Similarly, all of the stability pool samples achieved improved recoveries when extracted with ascorbic acid, regardless of the concentration utilized. While the addition of any ascorbic acid clearly increased olanzapine recoveries, no optimal ascorbic acid concentration could be discerned from the data.

In conclusion, adding ascorbic acid to blood specimens prior to storage appears to stabilize olanzapine. This practice, however, may not be practical for forensic specimens. A statistically significant amount of oxidized olanzapine can be recovered when specimens are extracted with ascorbic acid and analyzed as soon as possible.

Key words: Olanzapine, Oxidation, Forensic

P26

In vitro Production of an Unusually High Ethanol Concentration in a Postmortem Blood Specimen

Logan B. Umberger*, *Judith Tobin and Rebecca A. Jufer. State of Delaware Office of the Chief Medical Examiner, 200 S. Adams Street, Wilmington, DE 19801*

Ethanol is the most prevalent drug in our society and the most frequently analyzed drug in postmortem laboratories. It is well documented that postmortem blood ethanol concentrations can be affected by several factors including the site of blood sampling, the condition of the specimen, the presence of microorganisms in the specimen, the addition of microbial inhibitors to the specimen and the temperature of storage.

A case was received in our office involving a 59-year-old female with an extensive medical history who was found unresponsive. Initial investigation revealed a history of hypertensive cardiovascular disease and type 2 diabetes mellitus. The decedent's heart blood was submitted to the toxicology laboratory for analysis. The specimen was stored at 4°C without the addition of preservatives. A protein free filtrate was analyzed for alcohol on a gas chromatograph equipped with a flame ionization detector. The initial blood alcohol determination revealed an ethanol concentration of 0.064 g/dL and the presence of acetone. A repeat analysis of this specimen three days after the initial analysis resulted in an ethanol concentration of 0.150 g/dL. To resolve the discrepancy, a third analysis was performed and the ethanol concentration had increased to 0.227 g/dL. A final analysis twelve days after the initial analysis resulted in an ethanol concentration of 0.357 g/dL. Analysis of additional postmortem specimens for ethanol was not possible in this case, as heart blood was the only specimen submitted for toxicological analysis. In an attempt to resolve this issue, the decedent's medical history was closely examined. It was revealed that the decedent was a non-compliant diabetic. Her Accu Check® blood glucose monitor indicated that her last 2 blood glucose readings were 630 mg/dL and 698 mg/dL. It is suspected that the large amount of glucose in the decedent's blood specimen and the lack of microbial inhibitors added to the specimen created conditions that were favorable for the formation of ethanol. A substantial increase (0.293g/dL) was observed in the blood ethanol concentration over a period of 11 days. Had additional information not been learned during the investigation, the blood ethanol determination may have suggested that the decedent was quite intoxicated at the time of her death. This case illustrates the importance of obtaining a thorough case history, properly preserving postmortem specimens with microbial inhibitors and collecting multiple specimens for the postmortem determination of ethanol.

Key Words: Ethanol, Postmortem, Formation

P27

Development of an Integrated Oral Fluid Collection and Testing Drug Screen Device

Raphael Wong*, *Phung Nguyen, Baguio Wong and Dequn Wang. Branan Medical Corporation, Irvine, CA, U.S.A.*

Oral fluids have been gaining interest as a matrix for abused drug testing. Advantages of oral fluids include: 1) collection from individuals under direct observation without undue embarrassment or invasion of privacy; 2) ease of collection; and 3) eliminate the possibility of adulteration. Moreover, this matrix is a better indication of recent drug use than urine. Hence, it is preferred in the roadside detection of driving-under-the-influence.

An oral fluid on-site detection device for abused drugs has been developed. It combines collection and testing in a single unit and consists of a cap and a plastic housing containing a collection pad and two lateral flow immunoassay test strips. The following six drugs are detected: THC at 20 ng/ml, Cocaine at 20 ng/ml, Morphine at 20 ng/ml, PCP at 4 ng/ml, Amphetamine at 50 ng/ml and Methamphetamine at 50 ng/ml.

To run the test, the device is uncapped to expose the collection pad which is inserted into the donor's mouth. After sufficient amount of oral fluids is collected, the device is removed and recapped. Test reactions are initiated automatically with results available within 10 minutes. For confirmation, the collection pad is detached and sent to a laboratory for analysis.

Forty normal samples and forty spiked oral fluids samples for each drug at various concentrations (25% below cut-off, 50% below cut-off, 75% below cut-off and 300% above cut-off) were tested with the device. The preliminary results are as follows:

% of cut-off	100% below	75% below	50% below	25% below	300% above
# of Sample	40	10	10	10	10
THC	40-	10-	10-	8-/2+	2-/8+
Cocaine	40-	10-	10-	9-/1+	1-/9+
Morphine	40-	10-	10-	9-/1+	1-/9+
PCP	40-	10-	10-	8-/2+	2-/8+
Amphet.	40-	10-	9-/1+	9-/1+	2-/8+
Methamphet.	40-	10-	9-/1+	9-/1+	2-/8+

ROC Analysis of these preliminary results shows sensitivity and specificity as follows:

Drug Test	Specificity %	Sensitivity %
THC	97.1	80
Cocaine	98.6	90
Morphine	98.6	90
PCP	97.1	80
Amphetamine	97.1	80
Methamphetamine	97.1	80

These results suggest that the integrated collection and testing device can be utilized to detect abused drugs in oral fluids.

Key words: Oral fluid, Drugs of abuse, Drug screening

P28

Validation of a Microtiter Plate ELISA for Screening of Postmortem Blood for Opiates

Philip Kemp*[1], *Gary Sneed*[1], *Tom Kupiec*[2], *and Vina Spiehler*[3]. [1]*Office of the Medical Examiner, Oklahoma City, OK;* [2]*Analytical Research Laboratories, Inc., Oklahoma City, OK;* [3]*Spiehler & Associates, Newport Beach, CA, U.S.A.*

The object of this study was to evaluate the suitability of the Neogen Corporation microtiter plate enzyme-linked immunoassays (ELISA) for screening opiates in post-mortem blood. Post mortem whole blood specimens were obtained from (give number) drug-involved deaths which had been screened and confirmed positive for opiates. Forty negative specimens were obtained from non-opiate-involved deaths. Specimens were tested using the Neogen Opiates Group microtiter plate ELISA assays. No matrix effects were found for whole blood in these assays and a dilution of 1:5 was chosen to facilitate pipetting and to bring the IC50 of the microtiter plate ELISA assay within the range of opiates and benzodiazepines encountered in medical examiner specimens.

Confirmation and quantitation of the opiates was done by gas chromatograph (GC) and gas chromatography/mass spectrometer (GC/MS) following extraction using both liquid-liquid and solid-phase techniques. A HP 5890/5970 GC/MS, a 6890/5973 GC/MS and a HP 5890 GC equipped with nitrogen-phosphorous detection were used for the various opiate confirmations and quantitations.

True positive, true negatives, false positives and false negatives were determined and graphed for the ELISA results against GC/MS, gas chromatography-NPD and case histories. From these graphs and the ROC curves the optimal cutoff for the Neogen Opiates Group ELISA was found to be between 20 and 50 ng/ml morphine equivalents. The Neogen Opiates Group ELISA had a sensitivity of 95.2% ± 2.7% and a specificity of 92.2 % ± 3.4% vs GC/MS at a cutoff of 20 ng/ml cutoff and a sensitivity of 88.8% ± 3.9% and specificity of 96.8% ± 2.1% vs GC/MS at a 50 ng/ml morphine equivalents cutoff. The results of this study include a large inter-assay %CV for estimated concentrations that demonstrates the unreliability of immunoassay screens for estimating blood concentrations. Using sensitivity, specificity and predictive value for comparing accuracy, we found that the Neogen opiate assay was comparable to or better than previously published microtiter plate screening immunoassays.

Key words: Immunoassay, ELISA, Opiates

P29

Unusually High Blood Concentration of Alprazolam along with Fentanyl in a Case of Driving Impairment

James C. Kraner*[1], *Susan A. Lewis*[1], *and George F. Jackson*[2]. [1]*Palm Beach County Sheriff's Office, West Palm Beach, FL, U.S.A;* [2]*National Medical Services, Willow Grove, PA, U.S.A.*

Among the more common findings in cases of driving under the influence of drugs (DUID) is the anxiolytic, alprazolam (Xanax®). Here we present a case of DUID with a blood alprazolam concentration that is significantly greater than that typically found in cases of driving impairment or even fatal overdose.

Briefly, at approximately 1:15 P.M., a 42-year-old man with a history of driving impairment was arrested after backing up into a parked car at a department store parking lot. The driver was not injured and the other car was unoccupied. The arresting officer noted that the subject appeared sleepy and his pupils were dilated. The subject was cooperative but had a poor memory of the incident.

No alcohol was suspected and this was confirmed by a subsequent breath alcohol analysis. The subject indicated to the officer that he was taking alprazolam for anxiety. In addition, he was also found to be wearing a fentanyl patch. A blood specimen was obtained approximately 3 hours after the accident. Comprehensive drug screening established the presence of alprazolam. Directed analysis was then performed to quantitate alprazolam, fentanyl and their active metabolites using GC/MS and LC/MS/MS, respectively. Results are presented in Table 1.

Table 1

Alprazolam	α-Hydroxyalprazolam	Fentanyl	Norfentanyl
870 ng/mL	< 50 ng/mL	1.1 ng/mL	0.50 ng/mL

The therapeutic range for alprazolam is 10-50 ng/mL with the concentration being proportional to dose (1). Several studies have been published with extensive data on alprazolam in cases of driving impairment. In one study of 102 drivers arrested for DUID in which alprazolam was present, the average blood concentration was 119 ng/mL with a range of 8-642 ng/mL (2). A more recent study conducted of 82 cases positive for alprazolam found an average of 87 ng/mL and a range of 15 - 300 ng/mL (3). In reviewing the literature, the present case is one of the highest blood concentrations of alprazolam that we could find, including fatal overdoses. The lack of a higher α-hydroxyalprazolam level suggests that the suspect may not have been using excessive amounts of the drug on a chronic basis, prior to the incident. This is in contrast to what would be expected for an individual who could survive such a high alprazolam concentration, presumably due to $GABA_A$ receptor-mediated tolerance. An additional consideration of the circumstances of this case is the possibility that the subject intended to commit suicide. However, with the exception of the drug results, no other features of the case support this possibility.

References

1. Tietz, N.W., Clinical Guide to Laboratory Tests, 3rd ed., W.B. Saunders, 1995.
2. AAFS Drugs and Driving Committee Report, 1993.
3. Jackson, G.F., A Retrospective Analysis of DUI Drug and Alcohol Concentrations from a Large Suburban County, Annual Meeting of the Society of Forensic Toxicologists, New Orleans, LA, 2001.

Key words: Alprazolam, Impairment, Overdose

P30

Comparison of Cozart Oral Fluid Opiate ELISA and GC/MS Results Following Controlled Administration of Codeine Sulphate

Allan J. Barnes*[1], *Insook Kim*[1], *Raf Schepers*[1], *Eric T. Moolchan*[1], *Jon Oyler*[1], *Lisa Wilson*[2], *Gail Cooper*[2], *Claire Reid*[2], *Chris Hand*[2] *and Marilyn A. Huestis*[1]. [1]*Chemistry and Drug Metabolism, IRP, NIDA, NIH, Baltimore, MD 21124, U.S.A.,* [2]*Cozart Bioscience Ltd, Oxfordshire, OX144RU, UK*

The objective of this study was to evaluate the sensitivity, specificity and efficiency of the Cozart Opiate Microplate EIA Oral Fluid assay for detecting codeine use. For this study, the commercially available opiate kit was modified to optimize detection of codeine by substituting codeine calibrators (1 – 50 ng/mL) for the morphine standards. Semi-quantitative immunoassay results were compared with gas chromatography-mass spectrometry (GC/MS) results. Saliva or oral fluid testing is advantageous due to a less invasive specimen collection and reduced opportunity for specimen adulteration. These advantages are of interest to both drug treatment and drug testing programs. Oral fluid specimens (N=1406) were collected from nineteen (12 male, 7 female) healthy volunteers with a history of opiate abuse. The volunteers (15 African-American, 2 Caucasian, 2 Hispanic) provided informed consent to participate in an oral codeine sulphate administration protocol. They received three daily 60mg/70kg codeine sulphate doses, given every other day, and after a four week interval, three 120mg/70kg doses in the same manner. Oral fluid specimens were stored at –20 °C until analysis for opiates (codeine, norcodeine, morphine, normorphine) by solid phase extraction followed by GC/MS. The GC/MS limits of detection and quantitation were 2.5 ng/mL for all analytes. With the 2.5, 30 and 40 ng/mL GC/MS cutoffs, 26%, 19% and 18% of the oral fluid specimens were positive. To be considered positive, the concentration of any of the four analytes had to be equal to or exceed the respective cutoff. Interestingly, no morphine or normorphine was detected in oral fluid by GC/MS following oral codeine administration. Five screening/confirmation cutoff criteria were evaluated 2.5/2.5, 5/2.5, 10/2.5, 30/30 and 40/40 ng/mL. Calculations for sensitivity, specificity and efficiency were determined from the number of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) results at each screening/confirmation cutoff.

The sensitivities, specificities and efficiencies for the 2.5/2.5 cutoffs were 90.6%, 88.8%, and 89.3%; for 5/2.5 cutoffs 83.3%, 94.7%, and 91.7%; and for the 10/2.5 cutoffs 72.3%, 98.1% and 91.2%. As expected, as the screening cutoff increased, sensitivity decreased and specificity increased. Currently in the UK, the Forensic Science Service is using an oral fluid opiate screening and confirmation cutoff of 30 ng/mL. Application of these cutoffs with our data yielded sensitivity, specificity and efficiency results of 79.7%, 99.0%, and 95.4%. SAMSHA has proposed the use of a 40 ng/mL opiate screening and a 40 ng/mL codeine or morphine confirmation cutoff for the detection of opiate use. Sensitivity, specificity and efficiency were 76.7%, 99.1% and 95.1% when applying the SAMHSA criteria. These data indicate that the Cozart Opiate Microplate EIA Oral Fluid Assay can efficiently detect oral codeine use. In addition, these oral fluid data, collected following controlled oral codeine administration, may aid in the interpretation of opiate oral fluid test results and in the selection of appropriate oral fluid screening and confirmation cutoffs.

Key words: Oral Fluid, Opiates, ELISA

P31

Validation of a Microtiter Plate ELISA for Screening of Postmortem Blood for Benzodiazepines

*Lacinda DeCicco[1], Philip Kemp[2], Gary Sneed[2], **Thomas C. Kupiec***[1], Vina Spiehler[3]. [1]Analytical Research Laboratories, Inc., Oklahoma City, OK; [2]Office of the Medical Examiner, Oklahoma City, OK, [3]Spiehler & Associates, Newport Beach, CA, U.S.A.*

The purpose of this study was to evaluate the suitability of Neogen Corporation's Benzodiazepines Group microtiter plate enzyme-linked immunoassays (ELISA) for screening of benzodiazepines in postmortem blood. Fifty-three post-mortem whole blood specimens were obtained from drug-involved deaths, which had been screened and confirmed positive for benzodiazepines. An additional forty specimens were selected from cases confirmed negative for benzodiazepines. No matrix effects were found for whole blood in these assays and a 1:5 dilution was chosen to facilitate pipetting and to bring the IC50 of the microtiter plate ELISA assay within the range of benzodiazepines encountered in medical examiner specimens.

The number of true positives, true negatives, false positives and false negatives at nine concentrations (10 ng/ml to 500 ng/ml temazepam equivalents) were determined from the ELISA results and graphed against GC/MS, gas chromatography-NPD and case histories. The results from these graphs, and the Receiver Operating Curves (ROC) curves obtained from plotting the sensitivity vs. (1- specificity) at each putative cutoff concentration were used to determine the optimal cutoff concentration of between 20 and 50 ng/ml temazepam equivalents for the Neogen Benzodiazepines Group ELISA. The Neogen Benzodiazepine Group ELISA had a sensitivity of 100% $\pm$1.29% and a specificity of 94.6% $\pm$2.9% vs. GC/MS at a cutoff of 20 ng/ml; and a sensitivity of 95.8% $\pm$2.5% and a specificity of 98.2% $\pm$1.8% vs. GC/MS at a cutoff of 50 ng/ml. The results of this study include a large inter-assay %CV that is consistent with those reported in the literature and further demonstrate the unreliability of immunoassays in estimating blood concentrations. This assay was very accurate in diagnostic sensitivity (true positive rate), diagnostic specificity (true negative rate) and predictive value for comparing accuracy, and we found the assay very comparable to or better than previously published microtiter plate screening assays.

Key words: Immunoassay, Benzodiazepines, Postmortem

P32

Choice of an ELISA Assay for Screening Postmortem Blood for Amphetamine and/or Methamphetamine

Thomas C. Kupiec*[1], *Lacinda DeCicco*[1], *Vina Spiehler*[2], *Gary Sneed*[3] *and Philip Kemp*[3]. [1]*Analytical Research Laboratories, Inc., Oklahoma City, OK;* [2]*Spiehler & Associates, Newport Beach, CA,* [3]*Office of the Medical Examiner, Oklahoma City, OK, U.S.A.*

The object of this study was to evaluate the suitability of the Neogen Corporation microtiter plate enzyme-linked immunoassays (ELISA) for screening of post-mortem blood for amphetamine and methamphetamine and to choose the more appropriate assay for screening. Forty-seven post-mortem whole blood specimens were obtained from drug-involved deaths, which had been screened and confirmed positive for methamphetamine and/or amphetamine. Eighty-five negative specimens were obtained from non-amphetamines-involved deaths of which seventeen were decomposed. Specimens were tested using the Neogen Amphetamine Ultra and Neogen Methamphetamine/MDMA microtiter plate ELISA assays. No matrix effects were found for whole blood in these assays and a dilution of 1:5 was chosen to facilitate pipetting and to bring the IC50 of the microtiter plate ELISA assay within the range of amphetamines concentrations encountered in medical examiner specimens.

True positives, true negatives, false positives and false negatives were determined relative to GC/MS, gas chromatography-NPD and EIA and graphed for the ELISA. From these graphs and the ROC curves the optimal cutoff for the Neogen Methamphetamine /MDMA ELISA was 50 ng/ml methamphetamine equivalents. The optimum cutoff for the Neogen Amphetamine Ultra ELISA was 100 ng/ml amphetamine equivalents. The Neogen Methamphetamine ELISA had a sensitivity of 93.6% ± 3.5% and a specificity of 77.6% ± 4.5% vs. GC/MS at a cutoff of 50 ng/ml cutoff. The Neogen Amphetamine Ultra ELISA had a sensitivity of 95.7% ± 3.0% and a specificity of 72.9% ± 5.2% vs. GC/MS at a 100 ng/ml amphetamine equivalents cutoff. The areas under the ROC curve were equivalent for the two ELISA assays. The assays identified the presence of amphetamines in the post mortem blood specimens with less interference from sympathomimetic amines than the currently used microtiter plate EIA. These assays have precision and accuracy better than or comparable to the currently used EIA and previously published microplate screening immunoassays. The two Neogen ELISAs were equally accurate for identification of amphetamines in this sample population. The final choice of an assay will depend on the abuse profile in the screened population. If amphetamine is encountered as a drug of abuse of itself rather than just as a metabolite of methamphetamine, then Neogen Amphetamine Ultra assay would be the assay of choice. If the abuse profile of the screened population is methamphetamine and MDMA with amphetamine encountered mainly as a metabolite of methamphetamine, then the more precise and specific Neogen Methamphetamine/MDMA would be the assay of choice.

Key Words: Immunoassay, Methamphetamine, Amphetamine

P33

Driving under the Influence of Drugs (DUID) Cases in Virginia for 2001

*Randall P. Edwards, **Dwight D. Flammia*,** Julia M. Pearson and Joseph J. Saady. Virginia Division of Forensic Science, Richmond, VA*

The Code of Virginia mandates that the Virginia Division of Forensic Science (DFS) operate a centralized statewide system for driving under the influence of drugs (DUID), which includes ethanol (alcohol). As such, law enforcement personnel suspecting drug induced impairment transport the suspect to a facility capable of collecting a blood specimen (typically a hospital). The blood specimen is collected under chain of custody and forwarded via U. S. mail to the DFS Central Laboratory located in Richmond. In 2001, the laboratory processed 999 blood specimens for alcohol using gas chromatography headspace analysis. Specimens containing alcohol less than 0.09% by weight by volume were further screened for drugs of abuse (opiates, benzoylecgonine, benzodiazepines, cannabinoids, amphetamines and barbiturates) using fluorescence polarization immunoassay (FPIA). In the event of negative findings or findings below our specified concentration in the screen, the specimen undergoes more extensive gas chromatographic testing for prescription medications and other illicit substances (e.g. phencyclidine). Any positive finding requires confirmation by gas chromatography mass spectrometry (GC/MS) and quantitation. Findings are recorded on the certificate of analysis which are then forwarded to the court in the jurisdiction where the traffic offense occurred.

For the year 2001, we present data on all DUID blood specimens submitted in Virginia. Some of the most prevalent are included in the table below. A more extensive breakdown for all of the drugs found will be reported.

Drug	N	Average(mg/L)	Range(mg/L)
Alcohol only	421	700	100-4300
Alprazolam	167	0.09	0.02-1.4
Butalbital	57	8.0	1-21
Carisoprodol	49	6.1	2-15
Cocaine	64	0.05	0.01-0.3
Diazepam	90	0.3	0.1-1.3
Hydrocodone	54	0.06	0.02-0.6
Meprobamate	54	14	2-41
Morphine	9	0.11	0.01-1.9
Tetrahydrocannabinol	334	0.003	0.001-0.035

Key words: DUID, Impairment, Drugs

P 34

Opiate recidivism in a drug treatment program: Comparison of hair and urine data

Diana G. Wilkins*, *Bradley K. Charles, Jayme E. Day and Douglas E. Rollins. Center for Human Toxicology, University of Utah, Salt Lake City, UT, U.S.A.*

Monitoring recidivism by chemical analysis in drug treatment programs is a routine practice. The purpose of this study was to determine whether hair can be used as an adjunct specimen for the monitoring of opiate use in a treatment program. Subjects (n =10) initiating clinical therapy for opiate addiction were monitored for up to 17 weeks with hair and urinalysis. Questionnaires were administered weekly to document hair cuts and chemical treatments. Hair specimens were collected weekly by cutting at the scalp and segmented into 1-cm lengths prior to analysis. Urine specimens were analyzed by semi-quantitative radioimmunoassay (25 ng/mL cutoff). Codeine, morphine and 6-monoacetyl-morphine concentrations in hair were measured by LC/MS (20 pg/mg LOD).

Three of 10 subjects demonstrated little quantitative change in detectable analyte concentrations in hair segments closest to the scalp over time, suggesting continued opiate abuse. Urine opiate immunoassays were positive for all urines collected from these subjects (150/150) and were in good agreement with quantitative hair data.

For 2 subjects, drug concentrations measured in hair segments decreased over time to below the LOD/LOQ, suggesting discontinued/reduced opiate use. However, the frequency of positive urines (44/45 and 49/49) strongly suggested continued drug use and was in poor agreement with this hair data.

Three subjects demonstrated a decrease in hair drug concentrations to non-detectable concentrations by the end of the study, again suggesting discontinued/reduced opiate use. Urines collected were positive in an "intermittent" pattern throughout the study (26/42; 26/31; 34/37) for these subjects.

Finally, in 2 subjects drug and metabolite concentrations were either non-detectable or below the LOQ at the beginning of the study, despite substantial clinical evidence of drug abuse and positive urine opiate screens prior to enrollment. Analytes were not detectable in hair by the completion of the study, however urine specimens were occasionally (19/41) or frequently (41/53) positive throughout the study.

The potential effects of hair color and chemical treatment on quantitative hair data was considered. Subjects demonstrating good agreement between hair and urine data had dark brown or black hair and reported no chemical treatments before or during the study. Subjects with moderate to poor agreement between hair and urine data had various hair colors (blonde to dark brown). Some, but not all of these subjects reported using hair color (darker) during the study. Interestingly, the 2 subjects with low or non-detectable baseline hair concentrations reported using bleaching agents just prior to study enrollment.

Interpretation of the hair data in this study was difficult and generally was not in agreement with urine data in most cases. The effects of hair color and chemical treatments on interpretation of hair data requires further investigation. Urine appeared to be a more sensitive indicator of changes in the pattern(s) of drug use during the course of clinical drug treatment. This research was supported by NIH grant no. DA09096.

Key words: Recidivism, Hair, Opiates