AUTHOR INDEX
(**Bold** type denotes presenting author)

KEYWORDS

*******PLENARY SESSION*******

S1 Chromatography of Herbal Drugs on the MACRO! Scale: Challenges and Opportunities for Natural Products Chemistry

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The Natural Products Laboratory at RTI has a long history in the isolation and structure elucidation of novel compounds from nature, most notably the discovery of the anticancer agent, taxol. Recently, some of our research has focused on the evaluation of herbal drugs, especially the isolation of key compounds, both as reference standards and as study materials for preclinical development. This presentation will give some background into the goals of our research and then focus on a recent challenge, the isolation of gram-scale quantities of a series of natural product diastereoisomers.

Extracts of milk thistle (*Silybum marianum*), termed 'silymarin,' are used worldwide as non-prescription dietary supplements, primarily for purported hepatoprotective properties and recently as a chemopreventative for prostate cancer. Silymarin is a mixture of many compounds (at least eight), and the four major constituents are a group of structurally-related flavonolignans: silybin A, silybin B, isosilybin A, and isosilybin B. Our group was the first to describe the isolation of these diastereoisomers from each other, and as such, studies are underway to examine their respective activities vs preclinical animal models of human prostate cancer. The primary goal of this is to determine whether pure, isolated compounds exhibit greater efficacy and/or more selective biochemical actions than mixtures thereof. These studies also examine an ongoing debate in botanicals, namely, whether 'naturally-occurring' mixtures are superior or inferior to their purified, single-chemical entities.

To conduct such studies, large-scale samples of these difficult to isolate diastereoisomers were prepared. In particular, the isolation of isosilybin B (possibly the most active isomer, but present in the lowest amount) was rather cumbersome. A hybrid chromatographic/precipitative technique was developed, whereby mixtures were chromatographed at high concentrations, so as to induce formation of a precipitate in the column fractions. This precipitate was isolated to give samples significantly enriched in the desired compounds. By doing so, gram quantities of these compounds have been prepared, and in vivo studies are now underway.

Keywords: **Natural Products, Milk Thistle, Herbal Drugs**

*******2007 ERA RECIPIENT*******

S2 Analysis of Ayurvedic Medicinal Products for Heavy Metals Using a Direct Combustion Mercury Analyzer and Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

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Ayurvedic medicinal products are often employed as alternative treatments for several different ailments and have been used for medicinal purposes for centuries. Western societies are becoming more aware of these traditional methods of therapeutics due to globalization of the economy and the publication of medical studies. Ayurvedic natural health and herbal products are readily available in Canada and the United States. These products are easy to obtain from local health food stores, grocers, and online distributors, and are often much cheaper than modern medical treatments. However, numerous problems have been found in the quality of commercial herbal medicinal products. These problems include a lack of consistency of active ingredients, contamination with other herbs and toxins, and presence of heavy metals such as arsenic, lead, and mercury at unsafe concentrations in herbal medicines. In addition to exerting their own therapeutic effect, metals are intentionally added to some ayurvedic products to aid the drug in the formulation to reach its target site, and enhance the potency of the medication. At the present time, neither the prevalence of the metals nor the variance within the herbs is actually known. These problems are indicative of loopholes or laxity in the current regulations of India, Canada, and the United States. The regulation of herbal products falls under The Drugs and Cosmetics Act (DCA) of 1940 in India, The Food and Drugs Act (CFDA) of 1985 in Canada, and The Dietary Supplement Health and Education Act (DSHEA) of 1994 in the United States. Fever, illness, and death are obvious consequences of the acute or chronic use of such herbal products. It is clear that the widespread use of such possibly adulterated products has both clinical and post-mortem toxicological implications.

The objective was to analyze a variety of Indian ayurvedic medicinal products
that have recently been shown to contain high levels of heavy metals; specifically those that were available in the city of Surrey, British Columbia, Canada, for heavy metal content via ICP-MS. Mercury was analyzed by direct combustion of solid and liquid samples. Data was obtained for eight metals of interest: arsenic, cadmium, gold, lead, mercury, silver, thallium, and tin. Of the 70 samples tested, 10 were found to contain potentially harmful levels of arsenic, lead, mercury, and/or thallium. This represents 14% of samples that could possibly cause an adverse affect on the health of adults and/or children as per the established maximum daily intake limits for these metals. One product was found to be notably very high in arsenic content, containing 37 times the maximum daily allowable intake. On the other hand, cadmium, gold, silver, and tin were found to be present at negligible concentrations. From this project and recent studies, it is clear that the regulatory acts of India, Canada, and the USA need to be modified and refined for the strict monitoring of QA/QC practices employed in the herbal medicine industry. We agree with Saper et. al that the testing for heavy metals in herbal products be mandatory. We also propose that their oxidation states be known so that toxicity data may reflect the actual concentration of the dominant metal ion species. It is hoped that the findings of this project will serve to make the consumers of ayurvedic medicinal products more aware of the repercussions of possible ingestion of high levels of heavy metals and provide them with a means to understand the importance of published daily exposure limits of these metals.

Keywords: **ICP-MS, Ayurvedic, Metals**

*******2007 YSMA RECIPIENT*******

S3 Macronutritional Composition Induced Differential Gastrointestinal Absorption Kinetics of Alcohol: A Pharmacokinetic Analysis of Alcohol Absorption in the Postprandial State

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The current method of assessing gastrointestinal absorption of alcohol in the postprandial state include both the size of the meal as well as the time lapse since the meal was consumed relative to the ingestion of alcohol. This study suggests a modification to the current method by incorporating a third parameter—the glycemic index (GI) of the food—in assessing the gastrointestinal absorption rate k_a. In a two day study distinguished by the prandial state of the volunteers and separation by meal GI in the second day, volunteers assigned to the low GI group (protein) exhibited a 42% attenuation in both the peak breath alcohol concentration (BrAC) and total area under the curve (AUC) when compared to the volunteers assigned to the high GI group (carbohydrates) where only 13% attenuation in both peak BrAC and AUC was observed. Current analytical procedures that exclude the meal GI would predict both the peak BrAC and AUC to be consistent across all groups in the second study day while volunteers were in the postprandial state. These results warrant further studies with a larger sample size including gender and ethnic variation. Should the results of this preliminary study continue to be supported to such degree that was observed, modifications in predicting gastrointestinal absorption rates should be considered in pharmacokinetic analyses of alcohol in the postprandial state.

Keywords**: Alcohol, Absorption, Glycemic Index**

*******2007 YSMA RECIPIENT*******

S4 Pharmacokinetic Study of β-Hydroxy-β-methyl Butyrate in Male and Female Human Subjects

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Historically, athletes have used anabolic-androgenic steroids (AAS) in an effort to increase lean muscle mass and improve athletic performance. More recently, nutritional supplements containing other constituents, such as the essential amino acid metabolite β-hydroxy-β-methyl butyrate (HMB), are becoming popular alternatives to AAS. HMB has been suggested to protect against muscle damage after strenuous resistance exercise and help increase the gain of lean muscle mass. Limited data regarding the distribution of HMB in blood and urine is available. Therefore, the purpose of this study was to investigate the pharmacokinetics of HMB after single-dose (SD) and multiple-dose (MD) administration, as well as to determine the stability of HMB under various storage conditions.

After obtaining informed consent, male and female subjects (n=8) received both SD (3g) and MD (3g/day for 5 days) HMB in a cross-over design. Plasma was collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hrs; 24-hr urine collections were obtained at 0-24, 24-48, 48-72, 72-96, 96-120, and 120-144 hrs after the final dose of HMB. Quantitative determination of HMB in biological specimens was achieved following liquid-liquid extraction and analysis by gas chromatography/mass spectrometry with electrospray ionization.

The maximum plasma HMB concentration (C_{max}) was observed at 2 hrs following both the SD and MD protocols. The mean C_{max} (Mean $\pm$ SEM) for females was 45 ± 5 µg/mL (SD) and 58 ± 16 µg/mL (MD). For males, the mean C_{max} was 25 ± 5 µg/mL (SD) and 25 ± 5 µg/mL (MD). Following both the SD and MD protocols, the mean HMB plasma concentrations approached baseline values. Urine HMB concentrations ranged from 200-800 mg/day for all subjects, and returned to baseline by 24 hrs for both protocols. Data regarding the stability of HMB in biological specimens at three temperatures, across varying lengths of time, will also be presented. The initial data indicates that endogenous HMB values are < 2 µg/mL (fresh plasma) and < 10 mg/day (urine), but this is highly variable and may depend on dietary intake, as well as, degradation upon storage.

Keywords: **β-hydroxy-β-methyl butyrate, Pharmacokinetic, Human**

*******2007 ERA RECIPIENT*******

S5 Activity of Anabolic-Androgenic Steroids in a Biological Assay

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Anabolic-androgenic steroid (AAS) abuse is a growing problem not only among elite athletes but also in amateur, recreational, and adolescent athletes. There has been little investigation into the mechanism of action of AAS and there is also a need to develop new methods of detection. AAS activate the human androgen receptor (hAR), a transcription factor that, after it is activated by ligand binding, is shuttled to the nucleus of cells where gene transactivation occurs (which can be measured *in vitro* using a luciferase reporter gene assay). Although AAS have varying anabolic and androgenic properties, a relationship between AAS and subsequent activity has yet to be made. We hypothesize *AAS will increase the transactivation mediated through hAR in a dose-dependent manner*. To test this hypothesis, COS-7 cells were transfected with plasmids containing the hAR, an hAR specific promoter linked to firefly luciferase (PB-ARE2-luc), and renilla luciferase, as an internal control. These cells were then treated with varying doses of several commonly abused AAS (see Figure 1) or control for 24 hours. Following AAS treatment, cells were harvested and a luciferase assay was performed to measure the amount of gene transactivation mediated via the hAR. The doses of AAS used were 100nM, 50nM, 10nM, and 1nM. These doses range from very low to supraphysiological and encompass the normal physiological levels of androgens (between 15nM and 25nM). Our data show a dose-dependent response for the AAS studied. Figure 1 is a side by side comparison of the activity (potency) of a broad range of commonly abused AAS in a reproducible biological assay. NAN and OXA, potent synthetic AAS, show more activity than TEST, while EPI, COR and DEX show little activity. The endogenous androgens ANE and DHT show moderate activity. By assessing the transactivation in control (normal) and AAS abuser urines, it may be possible to determine the presence of previously non-detectable and designer AAS. If such a test can be modified to accept biological samples such as urine, it may become useful for detecting the abuse of AAS, as well as determining the relative anabolic potential of AAS as well as newly synthesized steroids and other new non-steroidal compounds.

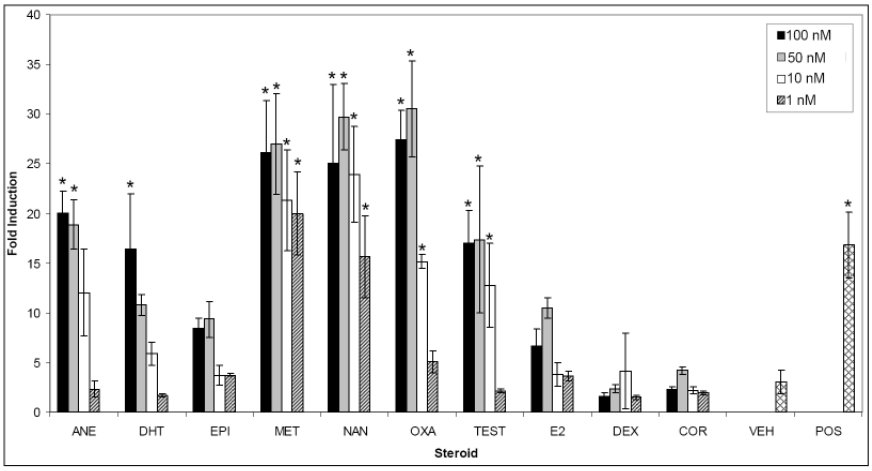

Figure 1. Fold induction of firefly luciferase mediated via the hAR in COS-7 cells after 24 hour AAS treatment. Values significantly different from vehicle (VEH) are indicated by *, p-value < 0.05. ANE, androsstenedione; DHT,dihydrotestosterone; EPI, epitestosterone; MET, methyltrienelone; NAN, nandrolone; OXA, oxandrolone; TEST, testosterone; E2, 17β-estradiol; DEX, dexamethasone; COR, cortisol; POS, positive control (100 nM TEST).

Research supported by NIH grants DA07820 and DK070060, and a University of Utah Funding Incentive Seed Grant

Keywords: **Anabolic Steroids, Androgen Receptor, Sports Doping**

S6 Driving Under the Influence - Toluene

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The abuse of volatile solvent compounds is commonly referred to as "huffing" the practice of which is regaining popularity. Typical abusers are introduced to the practice in their early teens and many will continue into adulthood. In 1995 almost one-quarter of teens had abused inhalants. Inevitably, some will drive under the influence of inhalants. In many jurisdictions, solvents are not covered under the DUI statues. In June 2007, the NY State highest court ruled that solvents are not specifically defined in the Driving While Intoxicated statute and therefore vehicular manslaughter charges would not hold up against a driver who abused just prior to a deadly accident. Several states, including Washington State are attempting to alter legislation to specifically included DUI solvents.

Subjects typically saturate a rag with the solvent, place it over the mouth, or inhale or spray the volatile substance directly into the mouth resulting in altered consciousness. Toluene is the most frequently abused inhalant, found in solvents, paints and other products. Onset of symptoms occurs within seconds to minutes following inhalation and produces an intense euphoria.

We report here a series of seven toluene impaired drivers who were evaluated by Drug Recognition Evaluation (DRE) officers. Blood toluene concentrations were determined by headspace GC, with headspace GCMS confirmation. The relative retention time (RRT) of toluene to the n-propanol internal standard is 3.77 and 2.84 on 2 different systems, (ethanol RRT is 0.61 and 0.57, respectively). In each case, toluene was the only impairing substance identified. All six subjects were males and their ages ranged from 25 – 56 (mean 36 years) and the blood toluene concentrations ranged from 12 – 45 mg/L (mean 25mg/L). The half-life of toluene in blood is 13 - 68 hours. A 1979 study of toluene abusers described significant signs of intoxication in subjects with blood concentrations of 1-2.5 mg/L. Half of those with blood concentrations between 2.5 – 10 mg/L were hospitalized for marked intoxication.

Two of the subjects we encountered were contacted after motor vehicle crashes. Four were stopped for severe erratic driving, and one for failing to stop at a red light. In all cases, impairment was very obvious; subjects had slurred speech, red, bloodshot watery eyes, appeared severely intoxicated. Solvent abuse was suspected for all subjects due to an obvious chemical odor. Two of the subjects had gold paint all over their face, hands and clothing. All but one subject were candid as to their methods and frequency of abusing the inhalant. For those who performed the DRE evaluation, there were inconsistencies on performance. Subjects generally did poorly on the walk and turn test. One subject was unable to keep his head still long enough to complete the HGN test; however the remaining six subjects had six of six clues present. Five subjects attempted the convergence test and all exhibited a lack of convergence. The results on the remaining tests were not consistent, for example 5 of 7 subjects completed the Romberg Balance test and of these, 3 exhibited fast internal clock, while 2 were very slow. Similarly, there were inconsistent observations on heart rate, blood pressure, pupil size and muscle tone. All subjects admitted to huffing in the car, and made statements which indicated that it was their practice to do so while driving, because the effects wore off rapidly.

This group is older than the stereotypical young adult inhalant abuser. The blood concentrations of these cases were much higher than earlier reports. This is consistent with longer term inhalant abuse and several of theses subjects did indicate they had been huffing for years. From the treatment literature, inhalant dependent adults have the poorest prognosis for recovery.

Keywords: **Driving Impairment, Toluene, Inhalant Abuse**

S7 Difluoroethane and Driving Impairment: "Dusted" in NorCal

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Inhalants, including halogenated hydrocarbon difluoroethane (commercially available "Dust Off"), although highly impairing, have been under-reported and infrequently prosecuted as agents of impaired driving. This is due to a number of issues, including: the typical pre and young teen demographic of abusers, the generally short half-lives associated with inhalants and analytical challenges arising from delayed and/or improper sampling, and analyte volatility. This case includes significant driving behavior, blood evidence obtained close to the time of driving, psychoactive parent drug quantitated in blood, driver admissions supporting impairment and a DRE *reconstruction* opinion. This case is not ideal as no DRE evaluation was performed, but DRE testimony was provided later via review of case facts.

A 34 year-old female presented with a qualitative blood confirmation of Pheniramine, an OTC antihistamine, and quantitative blood confirmation of 1.2ug/mL 1,1-Difluoroethane obtained one hour and 36 minutes after driving in northern California's Placer County at 9:14 am. The driving resulted in two back-to-back two-car collisions with blood obtained at a local ER, where defendant was transported for treatment of minor lacerations. Blood was negative for alcohol. The first collision resulted from the defendant racing thru a *Staples* parking lot, exiting onto the street without stopping and colliding into the rear of another vehicle. She continued onto the next intersection where she finally stopped for a red light. The driver of the impacted vehicle followed her, stopped behind and contacted the defendant. A confused conversation and unusual behavior from the defendant ensued. She continued onto the next intersection, where a second collision occurred. The defendant drifted into the right lane, at a high rate of speed (~ 55mph in a 35 mph zone), and collided with a second vehicle that was properly stopped at the intersection, causing both vehicles to spin around. This collision was witnessed by both the driver from the first collision and an off-duty County Sheriff's Deputy. The defendant's behavior included denials of collisions, multiple instances of non-responsive/unconscious appearance, a 10oz can of *Staples* brand compressed gas duster held between her legs, multiple attempts to continue driving following both collisions, and eventually resulted in the driver of the first impacted vehicle taking her car keys after witnessing the second collision.

The defendant admitted to use of DFE (just purchased) in the *Staples* parking lot, immediately preceding the first car collision. She stated that she had sprayed the propellant directly into her mouth one time and had no memory of either traffic collision. DFE effects are generally rapid acting and short lived, producing signs of CNS depression, including: headache, dizziness, incoordination, nausea, slowed reaction time, slurred speech, giddiness and unconsciousness. This is consistent with signs/symptoms noted for the defendant. It was later determined that the defendant was currently on probation for narcotics violations. She eventually plead guilty to DUID, following testimony of a CHP DRE at grand jury.

Keywords: **Difluoroethane, Inhalant Abuse, Driving Impairment**

S8 Inhalant Abuse and Driving Impairment

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The use of inhalants such as volatile solvents, fuels, aerosols, gases and anesthetics can result in serious driving impairment. The wide range of chemical, pharmacokinetic and pharmacodynamic properties, coupled with short half-lives in blood make detection of inhalant abuse in drivers challenging. Drug Recognition Experts (DRE) are trained to look for the physical indicators of impairment associated with inhalant abuse which can include horizontal and vertical gaze nystagmus (HGN, VGN), flushed facial skin tone, poor coordination, slowed reaction time, sedation and confusion. Inhalant abusers may also experience slowed pupillary reaction to light, increased pulse and flaccid muscle tone. Many factors including elapsed time from observed impairment to time of sample collection and laboratory capabilities make it difficult to corroborate documented impairment with meaningful blood toxicology results.

In this paper we present data from 10 cases of Wisconsin drivers suspected of driving under the influence of an inhalant. This study compares DRE evaluation data and blood toxicology results from those cases. Preserved whole blood specimens received by the Wisconsin State Laboratory of Hygiene (WSLH) from drivers suspected of operating under the influence of an inhalant are sent to NMS Labs, Willow Grove, PA, for quantitative inhalant testing. All 10 subjects in this study were males with a mean age of 26 years (range 16-49, median 21). The average amount of time from the initial contact with the arresting officer and the blood sample collection was 92 minutes (range 35-169, median 96). The mean elapsed time between blood sample collection and time of inhalant analysis was 44 days (range 16-180, median 20).

Five of the cases had positive toxicology results. Of those five, two of the subjects admitted to abusing paint and had positive results for acetaldehyde with one also positive for acetone and Methyl Ethyl Ketone. One subject admitted to abusing the computer cleaner Dust-off® and had a detectable amount of 1,1-difluoroethane. One subject, who admitted to inhaling oven cleaner, was found to have a trace amount of acetone. In the five cases with negative toxicology results four of the subjects admitted to abusing either paint, brake cleaner or gasoline, while no inhalant use information was available for the remaining subject.

Nine of the subjects exhibited indicators of impairment including HGN, slow speech and lack of coordination. One subject showed signs of impairment but did not exhibit HGN. The clinical indicators, psychological indicators and other signs of impairment will be discussed in detail. In all of the cases the DRE officers concluded that the subjects were impaired to the extent that they were unable to safely operate a motor vehicle.

The analytical challenges presented by inhalants often lead to a lack of correlation between laboratory results and DRE observations. Timely sample collection, information regarding the specific substance abused and proper toxicological testing procedures are all key factors in identifying inhalant use in impaired drivers.

Keywords: **Inhalant Abuse, Driving Impairment, Drug Recognition Expert**

S9 Case Study: A DUID Case Involving GHB

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In this presentation, attendees will be exposed to a case study involving drug impaired driving due to GHB intoxication. This presentation will impact the forensic community and/or humanity by demonstrating some of the impairing effects of GHB and give the audience a better understanding of the physical effects of this drug.

This case study involves a 41-year-old male who is involved in a collision where he subsequently fled the scene at low speed. The individual was pursued by multiple police cars but refused to stop for some distance. When the individual eventually stopped, he was in and out of consciousness, unable to speak or stand, and was soaked with sweat and urine. Due to his condition, he was transported by EMS and admitted to the hospital. An investigation of the vehicle at the scene recovered a small container of a clear liquid which was submitted to the Palm Beach County Sheriff's Office Crime Lab for analysis. Blood was also collected at the hospital approximately one hour after the incident and submitted to the PBSO Toxicology Unit for analysis. Analysis of the clear liquid indicated 1, 4-Butanediol and subsequent analysis of the blood indicated the presence of GHB at 159 mg/L. The individual was charged with misdemeanor DUID and, at present, the case is still pending.

Gamma-hydroxybutyric acid (GHB) is a central nervous system depressant first synthesized in the 1960's. GHB is a clear liquid slightly thicker than water with mild to no odor, although illicit production can produce significant odor. Analogs of GHB including GBL and 1,4 Butanediol are also significant because they are converted to GHB within the body. Over the years GHB has been used for induction of anesthesia and for treatment of alcohol and opiate withdrawal. Currently GHB is available as a treatment for narcolepsy under the brand name Xyrem. GHB has gained popularity as a common rave drug taken for its euphoric effects, by body builders to increase the release of growth hormone, and as a drug-facilitated sexual assault drug. Pharmacological effects of GHB are similar to alcohol including disinhibition, confusion, dizziness, and amnesia. Higher doses of GHB can lead to bradycardia, hypothermia, hypotension, hallucinations, somnolence, and loss of consciousness.

Keywords: **GHB, Driving Impairment**

S10 Los Angeles County: The Drugs that Drive Us

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Los Angeles County Department of Coroner, Los Angeles, CA, USA

California State Government Code 27491.25 requires that toxicology tests be performed on all traffic related fatalities. The law recommends that blood and/or urine specimens be tested for alcoholic contents, barbituric acid, and amphetamine derivative. Currently, the Los Angeles County Department of Coroner's Toxicology Laboratory routinely screens all traffic related fatalities for the presence of ethanol, marijuana, barbiturates, cocaine, methamphetamine, opiates, and phencyclidine. The literature suggests that sleeping medications, muscle relaxants, antihistamines, and other central nervous system (CNS) depressants have become more prevalent in traffic related fatalities.

This study was dual purpose: to assess expansion of the routine drug screen for traffic fatalities and to determine drug patterns of vehicle operators in Los Angeles County. Data from traffic fatalities in 2006 was evaluated for the presence of drugs, time of death, and location of death (scene vs. hospital). In addition to the routine drug screen, analytical tests were performed for benzodiazepines, carisoprodol, zolpidem, oxycodone, and other basic drugs (antidepressants, antihistamines, etc.). All drugs, except ethanol and other basic drugs, were screened via Immunalysis enzyme linked immunosorbent assays (ELISA). Alcohol was analyzed using Headspace Gas Chromatograph/Flame Ionization Detector (GC/FID). Basic drugs were screened utilizing a basic liquid/liquid extraction and then analyzed on both a GC/Nitrogen Phosphorous Detector (GC/NPD) and a GC/Mass Spectrometer (GC/MS).

In 2006, there were 421 traffic fatalities in which the decedent was the operator of an automobile, motorcycle, or bicycle and died within 24 hours of the accident. The following table represents the most commonly detected drugs, the number of positive cases, the percent drug prevalence, respective blood level ranges, and other information of interest.

Drug	# Cases	%	Range	Other Information
Ethanol	142	34	0.02 – 0.36 g/dL	110 (26%) ≥ 0.08g/dL
Cocaine	19	5	<0.03 – 6.0 µg/mL	11 (3%) Cocaethylene present
Benzoylecgonine	29	7	0.04 – 9.6 µg/mL	---
Methamphetamine	44	10	<0.03 – 6.5 µg/mL	---
Amphetamine	32	8	0.03 – 1.3 µg/mL	2 Amphetamine only
Marijuana (THC)	53	13	2.2 – 222 ng/mL	---
THC-COOH	83	20	<5.0 – 659 ng/mL	---
Benzodiazepines	42	10	---	32 (76% died in the hospital)
Antihistamines	37	9	---	Diphenhydramine most common
Antidepressants	18	4	---	Citalopram most common

Barbiturates, carisoprodol, and zolpidem were each detected in less than one percent of all fatalities in the dataset, and phencyclidine was never detected. Interpretation of results was complicated by possible hospital administration of drugs; 198 (47%) died in the hospital. Approximately 60% of all fatalities occurring within 24 hours were positive for one or more non-hospital administered drugs, while 30% were positive for more than one drug. In conclusion, although a wide variety of drugs were detected, alcohol remains the most prevalent, followed by marijuana and methamphetamine. The presence of drugs (i.e. benzodiazepines and antihistamines) detected outside of routine screening procedures supports the expansion of analytical testing for traffic fatalities.

Keywords: **Drug Patterns, Traffic Fatalities, Los Angeles County**

S11 The Tissue Distribution of Fluoride in a Fatal Case of Self-poisoning

María A. Martínez[*1], Salomé Ballesteros[1], Francisco J. Piga[1], Carolina Sánchez de la Torre[1] and Carlos Cubero[2]
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The purpose of this paper is to report a case of fluoride poisoning along with a discussion of poisoning characteristics, analytical procedures, and a review of previous reports of fatal intoxications with analytical data. A case of suicidal ingestion of 40 mL of a rust removal agent containing hydrofluoric acid and ammonium fluoride by a 33-year-old white male is presented. He had an organic personality disorder with residual schizophrenia and previous suicide attempts with therapeutic drugs and cleaning products. At admission he presented with a Glasgow coma score of 3; third degree atrioventricular block and asystole. Resuscitation efforts were performed during which the patient suffered two episodes of ventricular fibrillation followed by asystole. In spite of advanced resuscitation efforts and the administration of calcium chloride, he died 2.5 hours after the ingestion. Analytical data in the hospital showed calcium levels of 3.1 mg/dL and metabolic acidosis. Internal findings were: erosive gastritis, brain edema, as well as pulmonary and hepatic congestion. Quantitation of fluoride was performed using an ion-selective electrode for the anion. Disposition of fluoride in the different tissues was: peripheral blood, 19.4 mg/L; urine, 670 mg/L; vitreous humor, 2.5 mg/L; liver, 40.0 mg/kg; kidney, 60.0 mg/kg; lung, 17.5 mg/kg; brain, 2.5 mg/kg; spleen, 30.0 mg/kg; bone, 0.5 mg/ kg; and gastric content 1120 mg/L (67 mg total). Validation of the analytical method was performed using different spiked tissues, in a range of concentrations from 2.4 to 475 mg/L or mg/kg, and submitting them to dilution (1:25) to avoid the matrix effect and to bring these concentrations to the range of the aqueous calibration curve (0.19-19 mg/L). Limits of detection and quantitation were 0.02 and 0.1 mg/L, respectively. The linearity of the method, for all studies tissues, was excellent, with r^2 values of 0.999. Accuracy and precision were within 10.5% and 5.7%, respectively. Fluoride analyses using the ion selective electrode are simple, sensitive, and rapid. This is the first case we are aware of that provides a complete tissue distribution study of fluoride after a human poisoning, including a validated analytical method. Based on the autopsy findings, patient history, toxicology results, and previously reported data the forensic pathologists ruled that the cause of death was due to a fluoride poisoning, and the manner of death was listed as suicide.

Keywords: **Fluoride, Self-poisoning, Tissue Distribution, Postmortem**

*******2007 YSMA RECIPIENT*******

S12 Aripiprazole Analysis and Tissue Distribution in Postmortem Specimens

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Aripiprazole (Abilify®) is an atypical antipsychotic medication approved by the FDA in 2002 for use in the treatment of bipolar disorder and schizophrenia. Little is known about the drugs mechanism of action; however, it displays partial agonist activity at the D2 and 5-HT1A receptors and an antagonist activity at the 5-HT2A receptors. Aripiprazole is available for oral administration in tablets of 2, 5, 10, 15, 20 and 30mg, orally disintegrating tablets of 10 and 15mg, and oral solution of 1.0mg/mL. The normal adult dose ranges from 10 to 30mg once daily. Reported steady state plasma concentrations range from 70 to 585ug/L, depending on the dose. A minimal amount of information has been published about the toxicological analysis of this drug, and even less in postmortem specimens.

Approximately fifty cases, from over a two-year period, were analyzed for aripiprazole regardless of mode of death. These cases were selected because the drug was listed in the decedent's prescription history. Aripiprazole was not listed as the sole cause of death in any of the cases studied.

A basic, liquid-liquid extraction using n-butyl chloride with an acid clean-up step was used to extract aripiprazole from the specimens. Confirmation and quantitation were performed on a LC/MS/MS with linearity achieved over a range of 20 to 1000ug/L. Central blood, peripheral blood and liver specimens were analyzed with the following results:

	Range (ug/L)	N
Central Blood	+<20 - 1999	21
Peripheral Blood	+<20 - 1632	19
Liver	0.83 – 17.3 (ug/g)	14

The average central blood to peripheral blood ratio was 1.1 (0.09 to 2.14, n=16). Stability of aripiprazole was determined for a single case (postmortem central blood preserved with sodium fluoride) over time periods of 30, 90 and 120 days at both refrigerated (4˚C) at frozen (-18˚C) without significant loss of analyte.

This study was intended to provide other toxicologists with postmortem aripiprazole concentrations and tissue distribution data for comparisons and evaluation of their own casework.

Keywords: **Aripiprazole, Postmortem, Tissue Distribution**

S13 Case Report: The Use of the Herbicide Diquat and its Possible Involvement in a Poisoning

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Diquat is a quaternary amine compound that is generally used to control the growth of unwanted plants and vegetation. Diquat is considered to be a less toxic analogue of the herbicide paraquat. Diquat is available as a single herbicide and in combination with other chemicals, for both commercial and home use. The clinical course in fatalities usually involve irritation or necrosis at the site of contact, progressive decrease in renal function, followed by uncorrectable changes in metabolic, neurologic and cardiovascular processes.

A patient presented to the hospital approximately 15-30 minutes after ingestion of 8-10 oz of Round-Up Concentrate Plus Weed & Grass Killer (glyphosate, isopropylamine salt 18.0%; diquat dibromide 0.73%) in an admitted suicide gesture. The patient was noted to be anxious, but alert and cooperative. The patient complained of a (mild) sore throat and was noted to have mild pharyngeal erythema. Vital signs were stable, and initial oxygen saturation was 95% on room air, and blood chemistries were normal with the exception of serum bicarbonate of 18 mmol/L. Within an hour of ingestion, the patient vomited twice. Approximately 18 hours after ingestion, the patient was noted to be hypotensive (BP 80/52) with left arm twitching and depressed mental status. Shortly thereafter, the patient's pulse became very weak, and was intubated and resuscitated. The patient went into cardiac arrest and was resuscitated an additional time, but remained severely hypotensive. The patient developed metabolic acidosis with evidence of multi-organ system failure. The patient later developed myocardial ischemia and pulmonary edema. The patient remained comatose and expired approximately 34 hours after ingestion.

Using a modified method by RM de Almeida et al (2007), serum diquat concentrations were determined by GC/MS. The pH of 0.5 ml aliquots of calibrators and specimens was adjusted with 1.5 ml of 0.1 M phosphate buffer pH 8.0, then reacted with sodium borohydride and incubated at 60 ^{0}C for a minimum of 10 minutes, to reduce the quaternary ammonium amines. Diquat was extracted from the calibrators and specimens by SPE, conditioned with methanol and phosphate buffer, and eluted with methanol. The eluate was evaporated under nitrogen and reconstituted with methanol and injected into the GC/MS. Diquat in the calibrators and patient's serum samples was identified and quantitated using a Shimadzu QP-2010 GC/MS system operated in SIM mode, with a DB-5 column (30m x 0.25 mm x 0.33 μm) and a 5 m guard column. The GC oven temperature was programmed from 140 ^{0}C, 0.1 min hold, to 260 ^{0}C at 20 ^{0}C/min. The ions monitored for diquat and paraquat (internal standard) were: *m/z* 190, 108, 135 and 192, 148, 134, respectively. The serum diquat concentrations upon initial admission and 31 hours post ingestion were 6.0 mg/L and 1.7 mg/L, respectively.

Keywords: **Diquat, GC/MS, Round-Up**

S14 Clenbuterol-Tainted Heroin Detected in a Series of Cases from the Philadelphia Medical Examiner's Office

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The presence of clenbuterol, a potent beta2-adrenergic agonist, is reported in a series of cases in which the cause of death was attributed to illicit drug use. Clenbuterol is banned for use in treatment of humans in the United States. It is capable of exerting a variety of neurological and cardiovascular effects including: increase in aerobic capacity, oxygen transportation and blood pressure, CNS stimulation and relaxation of smooth muscle. Clenbuterol has been shown to increase the rate at which both fat and protein are metabolized while at the same time reducing the rate at which glycogen is stored. The primary usage of clenbuterol is in veterinary medicine as a bronchodilator (Ventipulmin®). It is the only member of its class to be approved by the US Food and Drug Administration for treatment of horses. However, because of its positive effect on respiration and subsequently on racing performance, the Association of Official Racing Chemists lists clenbuterol as a doping agent; its identification in post race samples may lead to sanctions.

Fluoridated bloods (1% NaF) and urines were collected during autopsies done between January 1 and March 31, 2007. In cases where blood or urine was not provided, either decomposition fluid or fluid from spleen was analyzed. Specimens from cases previously testing positive for opiates or fentanyl by either a broad-spectrum GC/MS analysis or enzyme linked immunosorbent assay (ELISA) then were screened specifically for clenbuterol using ELISA methodology. Specimens positive for clenbuterol were subsequently confirmed by GC/MS. Clenbuterol was extracted from positive specimens using solid phase extraction, derivatized using trimethyboroxime and analyzed utilizing electron impact GC-MS in the selected ion monitoring mode.

A total of 575 cases were screened in Toxicology during this three month period; the cause of death was determined by the medical examiner to be drug related in 106 cases. Clenbuterol was confirmed in ten of these cases. For nine of the decedents who were positive for clenbuterol, the cause of death was drug related; the tenth decedent was the victim of a homicide but had a history of heroin abuse. In each of the ten cases positive for clenbuterol, heroin use was confirmed either by the presence of 6-acetylmorphine or the presence of morphine with a history of heroin abuse. The highest concentration of clenbuterol found in blood specimens was 76 ng/mL.

The detection of clenbuterol was an unexpected finding since its use in the United States is restricted to veterinary medicine. In each of the nine cases in which the cause of death was attributed to illicit drug use, either heroin or cocaine or both illicit drugs made major contributions. However the influence of clenbuterol must also be considered since it is a potent beta agonist that may exert multiple adverse effects. Clenbuterol may be present in very low concentrations and therefore not detected by laboratories that do not routinely test for it. Emergency room physicians and toxicologists need to be aware of this when treating a suspected heroin user who presents atypically.

Keywords: **Clenbuterol, Heroin, Adulterants**

S15 Toxicology and National Association of Medical Examiners' Recommendations in Manner of Death Classifications in Suicide

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Suicide is a manner of death (MOD) frequently confronting medical examiners; in drug-related cases, a toxicologist is often requested to opine as to the relevance of the findings. When little more than an informed guess or speculation abounds, accident or undetermined are the modes most often invoked. The objectives of this presentation are to report the toxicological findings in three cases of suicide, acquaint toxicologists with the principles and guidelines described in *A Guide for Manner of Death Classification* (NAME, 2002), and discuss the data with respect to the perspective classification recommendations. The concepts of ***intent***, "beyond any reasonable doubt", "more likely than not" and "preponderance of evidence" will be discussed.

Case # 1 is an eighty-three year old male found in a vehicle within a closed garage. The key in the ignition switch was determined to be in the on the position. The decedent was known to be despondent over the recent death of his spouse. Case # 2 is a thirty-five year old male, with a history of intravenous drug abuse, found dead in a motel room with a plastic bag over his head. A "farewell note" was present. Case # 3 is a fifty-three year old male found dead at home with an accompanying history of ethanol, heroin and methadone use. The significant postmortem toxicological results are summarized in Table 1.

Table 1. Toxicological Findings by Case

Case 1	COHb by CO-Oximetery 41% Hydromorphone 0.36 mg/L (Femoral Blood)
Case 2	6-AM Present, LT 0.01 mg/L, Morphine 0.89 mg/L (Femoral Blood) Cocaine ND at 0.05 mg/L, Benzoylecgonine 0.09 mg/L (Femoral Blood) Sertraline, Norsertraline, Nordiazepam and Papaverine present (Heart Blood)
Case 3	6-AM 0.31 mg/L, Morphine 0.43 mg/L, Codeine 0.02 mg/L (Femoral Blood) Methadone 8.8 mg/L (Femoral Blood) Alprazolam 0.86 mg/L (Femoral Blood)

Conclusion: Postmortem toxicological examinations are integral to certifiers of death in uniformly applying NAME recommendations when ascribing MOD. Appropriate toxicology can contribute to more consistent MOD classifications rendered.

Keywords: **National Association of Medical Examiners (NAME), Manner of Death**

S16 Evaluation of Cocaine Cutting Agents in Drug Seizures, Antemortem, and Postmortem Cases in NE Ohio, 2005-2006

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Adulterants and diluents are routinely detected in drug seizures. These compounds are not typically reported in clinical or postmortem cases unless they are of pharmacological significance. In this study the authors compared and contrasted the incidence of two recently reported agents, diltiazem and levamisole, in drug seizures and toxicological specimens in adjoining counties over a two year period. All drug seizures positive for cocaine received for analysis by Lake County Crime Laboratory for 2005 and 2006 were reviewed. Analysis of these specimens included common adulterants but not diltiazem. In addition, all postmortem cases received for analysis at the Office of the Cuyahoga County Coroner (CCCO), Cleveland, OH, which were positive for cocaine and/or metabolites in at least one matrix were reviewed. Since comprehensive toxicological testing was conducted on all autopsied cases, the incidence of additional compounds was easily achieved. Antemortem specimens assayed at CCCO in OVI (operating a vehicle under the influence) and probation cases received from local law enforcement were reviewed providing comprehensive toxicology was conducted. Specimens were assayed for cocaine and metabolites by gas chromatography/mass spectrometry (GC/MS) in SIM after solid phase extraction (limit of quantitation [LOQ] 10 ng/mL). Diltiazem was detected by basic liquid-liquid extraction followed by gas chromatographic nitrogen phosphorus detection with GC/MS qualitative confirmation in SCAN mode (LOQ 50 ng/mL).

Lake County Crime Laboratory received 436 cocaine positive cases in 2005, 76% did not contain common adulterants such as procaine or caffeine. Cases with levamisole comprised 2.3% (n=10). In 2006 this increased to 5% or 25 cases (total n=505).

In comparison, in 2005, there were 166 postmortem cases positive for cocaine and metabolites. Diltiazem was detected in the urine of 2 cases (1.2%). The blood concentration range of cocaine and metabolites in these cases were as follows (ng/mL) [the two diltiazem cases are highlighted: 13-**35-344-**1429 cocaine (n=62), 16-**487-955**-8202 benzoylecgonine (BE) (n=126), and **21-**26-**132-**920 cocaethylene (CE) (n=16). Levamisole was detected in the blood in 1 case (0.6%), with a cocaine concentration of 588 ng/mL and 9919 ng/mL BE. The prevalence of these compounds in antemortem specimens (n=23) was 1 case positive for levamisole (4.3%). The cocaine and metabolite range in these specimens were as follows, ng/mL [levamisole case is highlighted] 23-**110**-390 cocaine, 41-**4382-**5716 BE.

In 2006 there were 217 postmortem cases positive for cocaine /metabolites with blood concentrations of cocaine 16-8119, BE 16-18825, and CE 19-2918 ng/mL (n=195). Diltiazem was detected in 14 cases (6.4%). Diltiazem was detected in the blood of 2 cases (at 50 and 280 ng/mL). The first case (50 ng/mL) contained a cocaine concentration of 54 ng/mL, and 2,515 ng/mL of BE. In the second case (280 ng/mL) no cocaine was detected, however, BE was found at a concentration of 331 ng/mL. Levamisole was detected in 7 cases (3.2%). The prevalence of these compounds in antemortem specimens (n=30) was 2 cases positive for levamisole (6.7%) and two containing diltiazem (6.7%). The cocaine / metabolite range in the antemortem cases was 55-**110**-1238 cocaine, 23-**4382** BE, 27-45 CE.

This study demonstrated that the incidence of levamisole and diltiazem increased from 2005 to 2006 in all case types. There appeared to be no relationship between the presence of these compounds and cocaine and metabolite concentrations. While it has been suggested that the addition of diltiazem to illicit cocaine may have a cardio-protective effect, the use of levamisole has yet to be elucidated.

Keywords: **Cocaine, Levamisole, Diltiazem**

S17 Evaluation of Urine Samples Utilizing Direct Analysis Real Time of Flight Mass Spectrometry **(AccuTOF™ DART™) for Postmortem Toxicology Screening**

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Objective: To minimize sample preparation and reduce analysis time and labor, a novel application of direct analysis in real time (DART) sample introduction coupled with time-of-flight (TOF) mass spectrometry was evaluated for analyzing urine samples at atmospheric pressure without the need for solvents or sample preparation.

Methods: Initially a mass spectral library was created using certified standards in solvent solutions. Then several drugs, including oxazepam and ibuprofen, were diluted in blank human urine and analyzed. The spectra indicated the presence of creatinine ($M+H^+$=114.058) and its dimer ($M+H^+$=227.116). At low levels, the drugs appeared to undergo ion suppression in part due to the presence of creatinine. To test this theory, the drugs were diluted at the same concentration in both urine and methanol. The spectra of the methanolic dilution resulted in the expected M+H ions, while those same ions were substantially reduced in urine. To determine if the results were pH dependent, separate solutions of creatinine in urine and water were made with pH ranging from acidic (~2) to basic (~10). The solutions were then spiked with drug and analyzed. While sensitivity was improved for compounds in water alone (without creatinine), the pH adjustments did not improve the drugs' detectability in urine. One strategy to address this was a liquid extraction into n-butyl chloride and subsequent concentration prior to analysis. This separated analytes of interest from creatinine and improved sensitivity.

Results And Discussion: The use of the AccuTOF-DART™ technology has the potential to positively affect productivity by allowing for minimal sample preparation and analysis in comparison to technologies currently used in postmortem laboratories. The presence of compounds such as creatinine appeared to impact the sensitivity of the DART source due to ion suppression and this effect is more substantial for some drugs (e.g. oxazepam) than for others (e.g. cocaine). However, more studies are currently being done to further evaluate its performance in screening for drugs in postmortem urine. To date, urine screening by TOF-DART appears to require some sample preparation to remove creatinine and achieve adequate sensitivity.

Keywords: **AccuTOF-DART™, Postmortem, Time of Flight Mass Spectrometry**

S18 Expanding the Capability of Drug Analysis in Hair Using a Simple, Common Extraction Procedure

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Background: The analysis of hair for an extended panel of drugs is the focus of this work. Prescription medications including tramadol, propoxyphene, fentanyl, oxycodone, carisoprodol, benzodiazepines and methadone are widely abused and cannot be detected by a routine hair-testing panel. However, many drugs can be analyzed in hair to help with forensic evaluation at autopsy, medical professional monitoring or non-regulated workplace testing.

Methods: A screening procedure for the detection of several medications in hair using ELISA was developed. Hair specimens were washed briefly with methanol and allowed to dry. Following cutting, they were placed in glass tubes and 0.025M phosphate buffer (0.5 mL) was added. The samples were sonicated at 75^{o}C for 2 hours. After 2 hours, 0.2 mL of supernatant was removed and 0.8 mL of bovine serum albumin (BSA) was added to dilute the aliquot 1:5. If necessary, the remaining extract could also be diluted and used in other ELISA assays. From the extract, a specific amount was used as the sample volume for the screening assays (Table 1). Since all immunoassays have a "sweet spot" (pg/well), a point at which the discrimination of the antibody is at its greatest, the specimen volume was calculated based on the desired cut-off (following literature review) and the point of highest discrimination. Presumptively positive samples (i.e. those with an absorbance lower than the cut-off concentrations) were taken forward to mass spectral confirmation using GC/MS or LC/MS/MS.

Cut-off concentrations for immunoassay screening and limits of quantitation

Drug	*Screening cut-off (pg/mg)*	*Sweet spot (pg/well)*	*ELISA volume (uL)*	*Confirmation technique*	*LOQ (pg/mg)*
Carisoprodol	1000	200 - 300	50	GC/MS	250
Oxazepam	200	40 - 80	50	LC/MS/MS	20
Fentanyl	20	4 - 8	75	LC/MS/MS	10
Methadone	200	50 - 150	65	GC/MS	50
Opiates	200	20 - 60	50	LC/MS/MS	50
Oxycodone	300	100 - 200	85	LC/MS/MS	50
Tramadol	1000	500 - 800	125	GC/MS	200
Propoxyphene	200	75 - 150	96	GC/MS	100

Summary: The use of hair for the detection of a wider range of drugs than is usually performed is described. The methods employ a relatively small amount of hair (10 mg), a simple, common extraction procedure, ELISA screening assays and mass spectrometric confirmatory methods. The procedures are being applied to various forensic areas including hair collected at autopsy. To date the results have correlated well, and the research is continuing.

Keywords: **Hair analysis, ELISA, Expanded Drug Profile**

S19 Comparison of Selected Drug Levels in Hair and Postmortem Specimens

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Hair specimens are collected routinely at autopsy on all cases at the Montgomery County Coroner's Office (MCCO). Hair may be used as a confirmatory specimen for those cases where routine specimens may be insufficient, too degraded, or absent. The pain management drugs, fentanyl, tramadol, oxycodone, methadone and propoxyphene are frequently encountered in case work at MCCO as are carisoprodol, meprobamate and the benzodiazepine class. Therefore a joint study between MCCO and Immunalysis was undertaken in order to compare the drug findings in autopsy specimens to their corresponding hair specimen and correlate that to case history when available. There were 37 cases included in this part of the study. None of the hair specimens were subjected to segmental hair analysis.

Screening for fentanyl, carisoprodol, meprobamate, methadone, benzodiazepines and oxycodone in blood or urine was accomplished by either ELISA or gas chromatography mass spectrometry (GC/MS) of the acidic, neutral or basic drug fraction. Propoxyphene and tramadol in blood or urine was screened by GC/MS of the basic fraction only. Quantitative measurement and confirmation of fentanyl and oxycodone in blood, liver or vitreous humor was accomplished using GC/MS with selected ion monitoring (SIM). The quantitative measurement and confirmation of propoxyphene, norpropoxyphene, methadone and tramadol in blood or liver was accomplished using gas chromatography (GC) with nitrogen phosphorus detection. Carisoprodol and meprobamate confirmation and quantitation were performed by GC with a flame ionization detector. The benzodiazepine class was confirmed by GC/MS operated in the SIM mode and quantitation was accomplished by GC with an electron capture detector.

Table 1. Cut-off concentrations for immunoassay screening and limits of quantitation in hair/ and in blood (bld)

Drug	*Screening cut-off concentration hair/bld pg/mg:ng/mL*	*Confirmation technique in hair only*	*Limit of quantitation in hair (pg/mg)*
Carisoprodol	1000/500	GC/MS	500
Benzodiazepines	200/10	LC/MS/MS	20
Fentanyl	20/1	LC/MS/MS	10
Methadone	200/25	GC/MS	50
Opiates	200/25	LC/MS/MS	50
Oxycodone	300/25	LC/MS/MS	50
Tramadol	1000/NA	GC/MS	200
Propoxyphene	200/NA	GC/MS	100

Table one lists the cut off levels for ELISA screening for blood established by MCCO and hair established by Immunalysis. There was good correlation for all cases between the autopsy specimen results and the hair results. When discrepancies did occur it could be explained by review of the case history or the elevated cut off levels in the hair screening as compared to the lower blood cut off levels. Hair analysis is used at MCCO for establishing historic drug use when necessary and also in decomposed bodies.

Keywords: **Hair, Pain Management Drugs, Postmortem**

S20 Development and Validation of a New LC/MS/MS Method for the Detection of *m*-, *p*- and *o*-Hydroxycocaine in Hair as an Indicator of Cocaine Use

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Background: There are some minor cocaine metabolites that are not found to be present in cocaine confiscations recorded by the DEA. The identification of these in vivo metabolites in aggressively washed hair establishes that ingestion of cocaine has occurred.

Objective: This laboratory investigated the possibility of determining several of these cocaine metabolites and their measurement in hair samples taken from criminal justice samples and in some previously known drug users' hair as part of a previous FDA study.

Methods: A LC/MS/MS method was developed to measure the presence of meta- (*m*-), ortho- (*o*-), and para- (*p*-) hydroxycocaine in hair. The *p*- and *o*- isomers represent true metabolites of cocaine, and m-hydroxycocaine has been determined to be a possible contaminant in confiscated cocaine by the DEA. ElSohly Laboratories kindly supplied the standards and internal standards used in this study. Analysis was performed on a triple quadrupole API 3000 Perkin Elmer Sciex MS equipped with an atomospheric pressure ionization source via an IonSpray (ESI). For LC, a series 200 micro binary pump with a Perkin Elmer Model 200 autosampler was used. The mobile phase was a mixture of water and acetonitrile containing 0.1% HCOOH. Ionization of analytes was obtained in positive mode. A high performance (HPLC) Betasil C8 column was used. The MS was operating in the multiple reaction mode (MRM). The instrument was set at unit resolution on both Q1 and Q3. For sample preparation, *m*-OH-coc-d3 internal standard was added to each sample. Linearity was demonstrated from 0.1ng/10 mg hair to 25 ng/10mg hair.

Results: The LC/MS/MS assay provides adequate sensitivity and reliability for the quantitative identification of the three isomers of hydroxycocaine at the proposed cutoff of 10 pg/mg (100 pg/10 mg hair or 0.10 ng/10 mg hair). Linearity, precision, carryover were determined. The use of LC/MS/MS afforded a complementary approach to our present LC/MS/MS method for cocaine, at the required sensitivity. The concentrations of the *p*-OH cocaine isomer were determined to be present at approximately 1% of the cocaine levels determined. The *o*-OH-cocaine was considerably less than 1%. Of the 275 samples studied, 217, 207 and 63 samples were positive at the proposed cutoff of 10 pg/mg for *m*-OH, *p*-OH and *o*-OH, respectively.

Conclusions: We recommend that *p*-hydroxycocaine at the proposed cutoff be added to the other metabolites currently being determined for the most accurate interpretation of hair analysis for cocaine. We conclude that this metabolite used with aggressively washed hair unequivocally distinguishes users from non users.

Keywords: **Hydroxycocaine, LC/MS/MS, Hair**

S21 Development and Production of Hair Reference Materials for Use as Control and Calibration for Hair Drug Testing

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Objectives: The Center for Forensic Sciences (CFS) at RTI International is now producing hair reference materials under an NIJ Award, 2006-DN-BX-K012, with objectives to improve the resolution and sensitivity of forensic analytical tools, as well as to enhance the productivity and portability of methods used in forensic laboratories.

Methods: CFS surveyed laboratories performing hair testing to determine which controlled substances are of most value to forensic laboratories and developed appropriate reference materials accordingly. Four reference materials at predetermined target concentrations are being produced in 2007. Some target concentrations are applicable to current screening and confirmatory testing, while others are for confirmatory testing only.
Head hair strands (14-20 g) were purchased, determined to be drug-free for analytes of interest, and washed to remove potential surface contaminants using an extended phosphate buffer wash. Darker hair samples that were not chemically treated and determined to be in good physical condition were utilized. Each reference material used hair from one individual. Fortification solutions were prepared with appropriate analytes and the intact hair strands were completely submerged in the solution at room temperature for a period of time that was concentration and analyte dependent. Aliquots were removed periodically during fortification process to test for analyte concentration. At the completion of the fortification process, hair was again decontaminated with an extended phosphate buffer wash. The hair was divided into 100 mg aliquots and placed in glass vials for storage.
The four reference materials and the theoretical target concentrations are as follows:

Reference Material 1	**Reference Material 2**	**Reference Material 3**	**Reference Material 4**
THCA (0.3 pg/mg	Morphine (500 pg/mg))	Cocaine (1500 pg/mg)	Amphetamine (750 pg/mg)
			Methamphetamine (750 pg/mg)
			MDMA (750 pg/mg)

Analyte concentrations in these materials will be verified through internal and external testing utilizing multiple forensic laboratories that routinely perform hair testing. Vials submitted to reference laboratories were chosen using a stratified random sampling scheme across the aliquoting process. These laboratories use standard testing procedures including extraction of drug analytes from hair matrix and analysis by GC/MS, GC/GC/MS, GC/MS/MS or LC/MS/MS.

Results: Preliminary results from the hair testing laboratories indicate that Reference Materials 1(THCA) and 2 (morphine) are within 20% of the theoretical target while Reference Materials 3(cocaine) and 4 (amphetamines) are substantially higher than the theoretical target. Reference testing and establishment of the uncertainty measurement are on-going and will be reported at SOFT.

Conclusions: The implementation of matrix-matched reference materials for hair at appropriate concentrations will further substantiate quality control measures of laboratories and improve the defensibility of their analytical results.

Keywords: **Hair, Reference Material, Drugs of Abuse**

S22 Confronting the Caffeine Confusion of Tea Infusions

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Caffeine is the world's most widely consumed drug. Although it is popular as a central nervous system stimulant, an excess of caffeine may be harmful to vulnerable populations such as children, pregnant women and individuals with common medical conditions (*e.g.*, hypertension and anxiety). Many individuals seek to reduce or eliminate caffeine from their diet; however, there is public confusion about the amount of caffeine in tea. For example, numerous Internet sites and blogs offer methods of tea preparation that claim to reduce caffeine content.

We have previously reported caffeine concentrations in a variety of beverages including specialty coffees, carbonated beverages and energy drinks. The focus of the present study was brewed teas. Twenty varieties of tea bags were selected to represent an assortment of white, green, black, decaffeinated and herbal teas from notable brands such as Bigelow, Lipton, Stash, Tazo, Twinings, and Two Leaves and a Bud.

Most individuals brew tea to their personal preference by varying the amount of water used, as well as the length of time the tea bag is steeped. To represent these variations, 20 varieties of tea bags were steeped for 1, 3 or 5 min in 6 oz of boiling water. In addition, 8 varieties of tea bags were steeped for the same length of time in 8 oz of boiling water.

Caffeine was extracted from the brewed tea using liquid-liquid extraction followed by gas chromatography with nitrogen-phosphorous detection (J. Anal. Toxicol. 27:520-522, 2003). The assay utilized linear calibration with concentration limits encompassing the wide range of caffeine concentrations present in brewed tea (e.g., 10-100 mg/L, 25-500 mg/L). Controls were included at multiple concentrations within these concentration limits.

Caffeine concentrations in white, green, and black teas ranged from 13 to 62 mg per serving with no observable trend in caffeine concentration due to the variety of tea. The decaffeinated teas contained caffeine less than 12 mg per serving, and caffeine was not detected in the herbal tea varieties. The brewing conditions affected the caffeine concentration of the tea. A 1 min steep time was not sufficient to remove all of the caffeine. For the 6 oz serving size, the mean extraction efficiency for the caffeinated teas was 60% compared to the 3 min steep time, with the exception of one tea which was 100% extracted at 1 min. The 3 min steep time resulted in a mean extraction efficiency of 87% compared to the 5 min steep time with the exception of one tea, which was 112%. For the 8 oz serving size, the mean extraction efficiency for the caffeinated teas was 78% for the 1 min steep time compared to 3 min, and 89% for 3 min compared to 5 min with the exception of one tea which was 108%. In most instances, the 6 oz and 8 oz serving sizes contained similar caffeine concentrations per oz.

These findings indicate that most brewed teas contain less caffeine per serving than brewed coffee. According to a previous study, caffeine concentration in specialty coffees ranged from 143.4 to 259.3 mg per 16 oz serving (J. Anal. Toxicol. 27:520-522, 2003). However, very few tea brands advertise the presence and/or content of caffeine. Of the tested brands, only Two Leaves and a Bud and Lipton mention caffeine in their package labels. Two Leaves and a Bud state that Organic Darjeeling contains less caffeine than coffee. Lipton reports concentrations of 55 mg/serving for its regular tea and 5 mg/serving for its decaffeinated tea, which were consistent with our findings.

Keywords: **Caffeine, Brewed Tea, Gas Chromatography**

S23 Whole Blood Cannabinoids in Daily Cannabis Users During Seven Days of Monitored Abstinence

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Δ9-tetrahydrocannabinol (THC) and its active metabolite, 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) are detected (≥1 ng/mL) in blood for several hours after a single, acute cannabis administration, while the inactive metabolite, 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) may be detected for several days. Few data are available on the duration of detection of these analytes in whole blood of daily cannabis users after cessation of cannabis smoking.

Methods: 28 daily cannabis users (aged 19-38, 50% male, 82.1% African American) provided written informed consent for this IRB-approved study, and resided on a closed clinical research unit for 7 days of drug abstinence. Whole blood was collected upon admission and once every 24 h thereafter. Cannabinoids were extracted by SPE (Clean Screen® ZSTHC020 extraction columns, United Chemical Technologies, Bristol, PA) and derivatized with BSTFA + 1% TMCS. Extracts were injected on an Agilent 6890 GC/5973MSD system (operated in EI/SIM mode). Two calibration curves (low, 0.125 – 25 and high, 25 – 100 ng/mL) were constructed with r^2 always > 0.99. The limit of quantification (LOQ) was 0.25 ng/mL for all analytes.

Results: Fifteen of 28 participants had whole blood free THC concentrations greater than the LOQ. Mean ± SD whole blood free THC, 11-OH-THC and THCCOOH concentrations were 1.1 ± 1.7 (N = 15, median 0.5, range 0.3 – 7.0), 1.1 ± 1.7 (N = 12, median 0.5, range 0.3 – 6.3) and 19.2 ± 20.4 (N = 28, median 14.7, range 2.8 – 91.7) ng/mL, respectively, upon admission. On day 7, concentrations were 1.3 ± 1.0 (N = 6, median 0.9, range 0.4 – 3.0), 0.6 ± 0.3 (N = 4, median 0.6, range 0.3 – 0.9) and 6.0 ± 8.4 (N = 28, median 3.2, range 0.4 – 36.5) ng/mL, respectively. In 3 of the 6 participants with measurable free THC on day 7, concentrations were above 1 ng/mL whole blood.

Conclusions: These are the first data on whole blood THC concentrations in chronic cannabis smokers detectable for multiple days after cannabis cessation and are important for interpretation of whole blood cannabinoid analyses in forensic investigations. Whole blood THC was found to be ≥ 1 ng/mL in three participants for 1 week after monitored cannabis abstinence. These data may reflect the accumulation of THC in the lipid compartment following daily cannabis smoking and its slow release back into the blood stream during cannabis abstinence.

Keywords: **Blood, Tetrahydrocannabinol, Driving Impairment, Cannabinoids**

S24 Detection Times of D^9-Tetrahydrocannabinol (THC), 11-Hydroxy-D^9-Tetrahydrocannabinol (11-OH-THC), and 11-nor-D^9-Tetrahydrocannabinol-9-Carboxylic Acid (THCCOOH) in Urine from Chronic Cannabis Users during Abstinence

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Cannabis is the most commonly abused drug world-wide. Due to the drug's high lipophilicity, body stores of Δ9-tetrahydrocannabinol (THC) increase during chronic cannabis use. Slow release of THC back into circulation is the rate-limiting step in its terminal elimination. In 1992, Kemp *et al* suggested that measurement of THC and its equipotent metabolite, 11-hydroxy-D^9-tetrahydrocannabinol (11-OH-THC), in urine suggested recent cannabis consumption. This approach could be highly useful for interpreting positive urine tests from driving under the influence, workplace, treatment and criminal justice cases. Cannabinoid excretion after chronic exposure has been an important aspect of our clinical research since 1990, but poses difficult logistical and study design issues. Today, we present clinical data from more than 15 years effort on the extended excretion of cannabinoids in urine and later this week, in whole blood.

Seven participants (aged 20-35, 57% male), who self-reported an extended history of daily cannabis use, provided written informed consent for this IRB-approved study. Subjects reported daily smoking of between one and five cannabis "blunts" prior to entering the closed research unit and initiating abstinence. Each urine specimen (n = 159) was collected individually *ad libidum*. Urinary cannabinoids were hydrolyzed by a tandem enzyme/alkaline method (*E. coli*, *β*-glucuronidase), extracted by SPE (Clean Screen® ZSTHC020 extraction columns, United Chemical Technologies, Bristol, PA), and derivatized with BSTFA. Trimethylsilyl derivatives of THC, 11-OH-THC, and 11-nor-D^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) were resolved and quantified in a 2-dimensional/cryotrap chromatography system on an Agilent 6890 GC/5973MSD system (operated in EI/SIM mode). Limit of quantification (LOQ) was 2.5 ng/mL for all analytes.

Mean ± SE time of last detection (> LOQ) of THC in urine was 9.0 ± 2.9 (range 2.9 – 24.7 days). THC maximum concentrations ranged from 4.6 – 14.8 ng/mL with a mean t_{max} of 0.7 ± 0.4 days of abstinence. THC detection rate through the last positive urine for each subject was 70.3%. Testing was discontinued when THC was no longer detectable (< LOQ) in three successive urine samples; therefore, last detection of 11-OH-THC and THCCOOH in chronic users remains to be determined; 11-OH-THC and THCCOOH were quantified in 98.7% and 100%, respectively, of these urine specimens. 11-OH-THC maximum concentration ranged from 25.4 – 132.8 ng/mL with mean time of highest concentration 1.0 days after cannabis cessation. 11-OH-THC and THCCOOH remained detectable for more than 25.0 days. THCCOOH maximum concentrations (range 117 – 594 ng/mL) were detected within 1.3 days (mean 0.6 ± 0.1 days) of initiation of abstinence.

These data indicate that following chronic cannabis smoking, THC, 11-OH-THC and THCCOOH can be measured in urine for more than 24 days, negating their value as biomarkers of recent cannabis use.

Keywords: **Tetrahydrocannabinol, Urine, Cannabinoids, Marijuana, GC/MS**

S25 Ethylglucuronide in Urine by LC-MS/MS with Human Excretion Profiles Following Dermal and Oral Ethanol Use

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Ethylglucuronide (EtG) is a direct alcohol biomarker and the U.S. Department of Health and Human Services has advised that specificity studies at low EtG levels are needed for distinction between alcohol exposure and alcohol consumption. We report the development and validation of a sensitive LC-MS/MS method for quantitation of EtG in urine, along with studies on the effect of both dermal exposure to ethanol-containing germicide and oral consumption of ethanol at varying doses. Analysis was performed by solid phase (aminopropyl) extraction of EtG and EtG-d_5, followed by liquid chromatographic separation on a HILIC silica column and negative electron spray interface with tandem mass spectroscopy. Transition ion monitoring of m/z 220.9 (EtG) and 225.9 (EtG-d_5) to 74.5 and 84.6 was performed with quantitation of m/z 74.5 ions. Validation studies were performed to determine LOD (10 ng/mL), LLOQ (25 ng/mL), pre-dilution linear range (25-10,000 ng/mL), and inter-assay precision (5-9% CV) at administrative thresholds of 100 ng/mL and 250 ng/mL.

In human studies, EtG levels ranged from <10–62 ng/mL in 25 urine specimens from subjects abstaining from ethanol use or incidental ethanol exposure for at least 5 days, indicating either unrecognized ethanol exposure or endogenous ethanol metabolism. During and five days following a five day use of hand sanitizer (20 washes/day with 60% ethanol-containing handwash), EtG concentration in 88 first-morning voids obtained from eight subjects ranged from <10 to 114 ng/mL. EtG excretion profiles with four subjects following a 24 gram ethanol drink showed maximum urine EtG concentration ranging from 12,200 to 83,200 ng/mL at 3 to 8 hours post-dose, along with EtG levels exceeding 150 ng/mL for 28 to 39 hours post-dose. Urine ethanol was undetectable (<10mg/dL) within 3-6 hours of the 24 gram dose. Individual subjects showed a dose dependent increase in the percentage of ethanol excreted as EtG with oral ethanol doses of 3, 6, 12, and 24 grams. The percentage of ethanol excreted as EtG with the 24 gram ethanol dose ranged from 0.041-0.083% as compared to only 0.005-0.027% with a 3 gram dose.

Conclusions: A sensitive EtG method has been developed and validated with human studies demonstrating 1) EtG is detectable at low concentration (<100 ng/mL) when ethanol use or other exposures are not evident, 2) EtG levels remain less than 150 ng/mL in first morning-void urine from subjects with repeated dermal exposure to hand sanitizer containing ethanol, 3) EtG levels exceed 150 ng/mL for up to 39 hours after a 24 gram ethanol drink, and 4) oral dosing results in a dose dependent increase in the percentage of ethanol excreted as EtG .

Keywords: **Ethyl Glucuronide, Urinary Excretion, LC/MS/MS**

S26 Suggesting a Cut-Off for Ethyl Glucuronide in Urine for Forensic Proof of Ethanol Consumption

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Objective: Ethyl glucuronide (EtG) is an ethanol consumption marker with a very high sensitivity and specificity. Nevertheless, SAMHSA recommended not to use EtG in forensic settings as it is not clear how unintended alcohol intake can affect urinary EtG concentrations. Meanwhile, Costantino [1] reported EtG positive results even after use of alcoholic mouthwash (15 minutes of gargling or use as intended by the manufacturer three times a day). Due to our experience from various drinking experiments, we recommend guidelines for a reasonable use of EtG as an ethanol consumption marker.
Methods: Urine samples were collected from drinking experiments with social drinkers. EtG was determined by use of an API 365 liquid chromatography – tandem mass spectrometer with a validated method, comprising protein precipitation with methanol, evaporation of the supernatant, reconstitution with LC-solvent, chromatographic separation on a 250 mm Polar RP column and mass spectrometric analysis.
Results: Drinking amounts in the experiments ranged from 4.5 L German beer (150 g ethanol) in 12.5 hours down to a single administration of 0.5 g of ethanol. EtG was detectable for up to 80 hours after the consumption of 150 g ethanol, up to 12 hours after the consumption of 1.0 g ethanol and up to 10 hours after the consumption of 0.5 g ethanol. The peak urinary concentrations showed great interindividual variations and ranged from < LOD (0.05 mg/L) to 0.35 mg/L after consumption of 1 g ethanol. After consumption of 0.5 g ethanol they were in the range of 0.06 – 0.15 mg/L.
Conclusions: Consumption of small amounts of ethanol is well detectable by LC-MS/MS. Testing is performed in several laboratories in Germany, Sweden, Norway, Italy, Luxembourg, USA and proficiency tests are provided and performed on a regular basis. Ethyl sulfate is a complementary and more stable marker than EtG, and can be analyzed in the same chromatographic LC-MS/MS run as EtG, however no SPE method has been established for preconcentration of EtS. Positive EtG results after "lack of alcohol consumption" should not be regarded as false positives, but as a proof for uptake of alcohol, possibly in small amounts which should not give reason to impose a sanction in some cases (e.g. work place drug testing in places where contact with alcohol containing lotions is not avoidable). If total abstinence is required, e.g. in alcohol withdrawal therapy, the person should not become only 'a little abstinent' from obvious alcoholic containing materials, but completely avoid contact to ethanol in all of its forms (e.g. mouthwash, communion wine, skin disinfectants, ethanol containing medication). This requires a competent instruction of the patient during therapy about which products can contain ethanol and though have to be avoided. On the other hand, the cut-off should be increased for forensic requirements - e.g. to 0.5 mg/L – using a normalization to 100 mg/dL creatinine. In our opinion, this includes a safe-zone that is large enough to avoid false-positives. With our results, exceeding the proposed cut-off would correspond with a consumption of more than 1 g of alcohol. We assume that this is an amount of alcohol that should be avoided by abstinent persons and cannot be taken up accidentally. In line with the 'morphine in poppy seed'-problem, one can always try to override the cut-off by inappropriate consumption of large amounts of analyte-containing (or analyte-creating) substances. [2] But this should rather be regarded as a problem in withdrawal therapy and a lack of education about ethanol traces in products of daily use. For proof of long-term abstinence, an additional hair-analysis for EtG and/or fatty acid ethyl esters (FAEE) should be performed.

Keywords: **Ethyl Glucuronide, Alcohol Consumption Biomarker, SAMHSA Recommendation**

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S27 Prevalence and Disposition of Drugs of Abuse and Opioid Treatment Drugs in Oral Fluid

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Testing oral fluid for drugs of abuse has been studied under many conditions but rarely has been evaluated in large population databases. We evaluated oral fluid tests in a database from a commercial laboratory in the United Kingdom composed of 8679 confirmed positive results. The results originated from 635,000 specimens collected over the period of May, 2004 through September, 2006. Oral fluid specimens were collected with the Intercept® oral fluid collection device, screened by enzyme immunoassay (EIA), and confirmed by GC-MS or GC-MS-MS. The database was organized by collection settings (legal/treatment, N = 8198 specimens; and workplace, N = 481 specimens) and by drug groups (without consideration of collection setting). The drug groups were as follows (number of screened and confirmed positives): Amphetamines (468); Benzodiazepines (892); Buprenorphine (276); Cannabinoids (725); Cocaine (1443); Methadone (998); and Opiates (5739). Analytes included in each group and their %cross-reactivity were as follows: Amphetamines: d-amphetamine (100), d-methamphetamine (100), MDMA (290), MDA (49); Benzodiazepines: nordiazepam (100), chlordiazepoxide (16), diazepam (<1), lorazepam (500), oxazepam (14), temazepam (<2); Buprenorphine: buprenorphine (100), norbuprenorphine (9); Cannabinoids: THC (100), cannabidiol (7), cannabinol (76), 11-HO-THC (590), THCCOOH (1600); Cocaine: BZE (100), cocaethylene (200), cocaine (64); Methadone: methadone (100), EDDP (<1); Opiates: morphine (100), 6-AM (65), codeine (>100), heroin (43), DHC (190). The goal of the study was to provide drug/metabolite prevalence data, concentrations, and drugs/metabolite patterns encountered in oral fluid. Comparison of results by collection setting indicated differences in relative frequency, primarily for opiates and cannabinoids. Opiate positives were most frequently observed for specimens collected in legal/treatment settings, whereas cannabinoids were most frequently reported in the workplace. An array of information on drug and metabolite occurrences and concentration arose from evaluation of the data by drug groups. Amphetamine was the predominant drug reported for the Amphetamines Group; approximately 10% were also positive for MDA and/or MDMA; and methamphetamine was rarely reported. Multiple combinations of diazepam, nordiazepam, oxazepam, temazepam, chlordiazepoxide and lorazepam were reported for the Benzodiazepine Group. Buprenorphine, an opioid treatment drug, was the predominant analyte reported, but low concentrations of norbuprenorphine were frequently reported. THC was the predominant analyte reported in the Cannabinoids Group and was frequently reported in combination with cannabidiol and cannabinol. THCCOOH was reported in only 12.5% of these specimens and was never reported in the absence of THC. HO-THC was reported in 5.7% of the specimens. In the Cocaine Group, cocaine was present, often in combination with BZE, but also as the sole analyte in 17.3% of the specimens. AEME and cocaethylene were reported in 10.4% and 5.5% of the specimens. Methadone, another opioid treatment drug, was reported in all specimens for the Methadone Group; EDDP was reported in 30.1% of the specimens. In the Opiates Group, morphine, codeine and 6-acetylmorhine were most frequently reported, often in combination. The frequency of detection of 6-acetylmorphine when morphine was present (N = 4575 specimens) was 77.5%. Surprisingly, heroin (19.0%; N = 1091 specimens) and 6-acetylcodeine (24.9%; N = 1431 specimens) were frequently reported. The results from analysis of this large oral fluid database offer a rich mixture of new information on detection frequency, drug and metabolite patterns, and concentration data on drugs of abuse.

Keywords: **Oral Fluid, Prevalence, Drug Abuse**

S28 Urine Drug Testing of Pain Patients: Licit and Illicit Drug Patterns

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Pain management is one of the most rapidly evolving fields of medicine. Millions of chronic sufferers now enter into "agreements" with treatment specialists to protect patient access to controlled substances and the physician's ability to prescribe them. As part of that agreement, patients may be subjected to urine drug tests (UDT). The detection of unauthorized substances may prompt referral for assessment for addictive disorder. The recent surge in the use of UDT in treatment clinics has emerged virtually without the benefit of toxicologic studies of this population. As part of an ongoing study of UDT in different populations, we assessed drug prevalence and disposition patterns in a database of confirmed positive results for 10924 pain patients (representing 31 clinics in 6 states over the period of Jan-Dec, 2006). All specimens were screened by immunoassay and confirmed by GC-MS or LC-MS-MS. The database was organized by drug groups (number of confirmed positives) as follows: Amphetamines (160); Barbiturates (308); Benzodiazepines (2397); Cannabis (967); Carisoprodol (612); Cocaine (310); Fentanyl (466); Meperidine (59); Methadone (1209); Opiates (8998); and Propoxyphene (385). The goal of this initial study was to provide drug/metabolite prevalence data, concentrations, and drug/metabolite patterns encountered within and between drug groups. Assessment of within drug/metabolite patterns provided the following information. Amphetamine (121) was the predominant drug reported for the Amphetamines group, followed by amphetamine/methamphetamine (38); phentermine (35); and MDMA/MDA (4). Butalbital (304) was predominant in the Barbiturates group followed by secobarbital (7), pentobarbital (3) and butabarbital (1). Multiple combinations of oxazepam, temazepam, desmethyldiazepam, alpha-hydroxyalprazolam, clonazepam, and lorazepam were reported for the Benzodiazepines group. THCCOOH was the only analyte tested in the Cannabis group and was present at a median concentration of 40 ng/mL (range 2.0 – 11554 ng/mL). Meprobamate was the predominant single analyte (337) reported in the Carisoprodol group, followed by carisoprodol/meprobamate (239), and carisoprodol (36). Benzoylecgonine was the only analyte tested in the Cocaine group and was present at a median concentration of 1043 ng/mL (range 50.0 - 425055 ng/mL). Fentanyl was the only analyte reported in the Fentanyl group and was present at a median concentration of 22 ng/mL (range 0.3 – 2382 ng/mL). For the Meperidine group, there was an approximate equal distribution of specimens positive for meperidine (19), normeperidine (20) and meperidine/normeperidine (20). Methadone in combination with metabolite (EDDP) was the predominant finding (981) for the Methadone group followed by EDDP (152) and methadone (75). Within the Opiates group, hydrocodone (5749) was the predominant analyte reported followed by hydromorphone (3699), dihydrocodeine (2286), oxycodone (2068), oxymorphone (1630), and morphine (1061). The most prevalent combinations within the Opiates Group were hydrocodone/hydromorphone (1227), hydrocodone/hydromorphone/ dihydrocodeine (1220), and oxycodone/oxymorphone (1107). Norpropoxyphene was the predominant finding (191) for the Propoxyphene group followed by propoxyphene/norpropoxyphene (156) and propoxyphene (38). Assessment of between drug groups provided an indication of the number of drugs used by the patient. Approximately 62% of the patients had used a single drug, 27% had used two drugs, 8% had used 3 drugs, 2% had used 4 drugs, 0.4% had used 5 drugs, and 0.05 % had used 6 drugs and 0.01% had used at least 7 drugs. There was evidence of possible illicit drug use based on positive tests for cannabis (8.9%), cocaine (2.9%), and ecstasy (<0.1%). These results provide a rich array of information to treatment specialists who practice compliance monitoring of pain patients. Clearly, a comprehensive UDT program requires the use of a broader range of highly sensitive assays than commonly used in traditional settings such as the workplace.

Keywords: **Urine Drug Testing, Pain Management Drugs, Compliance Monitoring**

S29 Performance of Drug Abuse Testing with POCT Devices and Onsite Instruments in Criminal Justice Settings

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The use of on-site or point-of-collection testing (POCT) methods for testing for drugs of abuse by arrestees, probationers and parolees has increased significantly in a wide variety of criminal justice settings. However, concern remains about the accuracy of such testing unless appropriate quality control and monitoring measures are in place. This paper presents an evaluation of real-world results for donor specimens and PT samples tested on-site by multiple criminal justice offices using various POCT devices and commercial analyzers with immunoassay reagents and submitted to a DHHS-certified laboratory for confirmation. Also, a description and summary of findings from on-site inspections of sites using POCT devices or instruments will be presented.

The confirmations were performed using GC/MS at the following cut-offs (ng/mL): benzoylecgonine 150, THC-9-acid 15, amphetamines 250, opiates 150, and PCP 25. Amphetamines included: amphetamine, methamphetamine, MDMA, MDA and MDEA, and opiates included: codeine, morphine, hydrocodone, hydromorphone and oxycodone. All specimens submitted for "confirmation only" were subject to GC/MS analysis regardless of any preliminary immunoassay performed by the confirmation laboratory.

The confirmation laboratory provided Duo Research quantitative GC/MS results for the period of October 1, 2005, through January 31, 2007, for 40,257 specimens submitted for confirmation. The analysis of the data was performed for all results for each drug class and selected submitting sites using POCT devices or instruments.

For all specimens submitted for cannabinoid analysis, 3,433 (20.7%) of a total of 16,579 specimens failed to confirm at the stated cutoff. Three regional instrumented sites had fail-to-confirm rates of 2.1 to 13.7%, whereas several large POCT sites had fail-to-confirm rates of 11 to 40%. For benzoylecgonine confirmations, 2,465 (22.6%) of a total of 10,920 specimens failed to confirm. Several of the larger instrumented sites had fail-to-confirm rates of 1.4 to 31.8%, whereas several large POCT sites had fail-to-confirm rates of 13.0 to 46.6%. For PCP, more than half, 481 (53.6%) of a total of 898 specimens failed to confirm. Instrumented sites had fail-to-confirm rates of 10.9 to 48.6%, and several POCT sites had fail-to-confirm rates of 40 to 79.6%.

The opiates as a class had 1,875 (23.7%) of 7,923 specimens submitted that had no concentration evident above the LOD for any of the five analytes. For specimens containing morphine as the predominate analyte, 218 (9.5%) of a total of 2,303 specimens failed to confirm. The other analytes had similar results.

The amphetamines as a class had 1,685 (42.8%) of 3,937 specimens submitted that had no concentration evident above the LOD for any of the five analytes. For specimens containing methamphetamine as the predominate analyte, 19 (1.1%) of a total of 1,780 specimens failed to confirm. The other analytes had similar results.

For selected sites, the on-site results from offices using instruments had better confirmation rates than offices using POCT devices. Specific examples will be presented with the variations and advantages and disadvantages of both methods.

As part of an ongoing monitoring program that includes the submission of proficiency test samples and evaluation of confirmation rates, periodic inspections of on-site testing facilities are conducted. The approach and findings will be presented.

Keywords: **Point of Care Testing, Criminal Justice**

S30 The Effects of Time, Temperature, and Various Physiological Conditions on the pH of Urine Specimens Collected for Workplace Testing

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Objective: The Mandatory Guidelines for Federal Workplace Drug Testing Programs require specimen validity testing, which includes pH, to identify those urine specimens submitted by donors attempting to suborn the testing process. Invalid categories relating to clinical characteristics of urine include pH ≥3 and < 4.5, pH ≥9 and <11, creatinine <2 mg/dL with acceptable specific gravity, and acceptable creatinine with specific gravity ≤1.0010 or ≥1.0200. Recently, a number of specimens with results within the upper pH invalid limits, typically in the range of 9.1 to 9.3, have been reported with no known evidence of donor tampering.

The study objective was to determine if these increased urine pH findings were the result of prolonged exposure to increased environmental temperatures during specimen standing or transport and/or to the impact of various physiological conditions, including pathological microbial contamination, glucosuria, and proteinuria.

Methods: A freshly collected, random (untimed) urine specimen pool was divided into aliquots that were either spiked with pathological levels of bacteria (*Pseudomonas aeruginosa*) (49,000 colonies/mL), yeast (*Rhodotonula minuta*) (49,000 colonies/mL), D-(+)-glucose (2,000 mg/dL), and human albumin (2,000 mg/dL) in various combinations or left unspiked. The aliquots were kept unpreserved at various storage temperatures (-20, 4, 25, 37, and 93 ^{o}C) for a maximum of two weeks. On each specified day (days 1, 2, 3, 7, 8, 9, 10, and 14 post-void), the pH of each aliquot was measured in duplicate using a pH meter.

Results: Increasing storage temperatures were found to be associated with the increasing urine pH, with the exception of those specimens containing glucose at 93 ^{o}C where the pH values decreased with time after day one. The pH values of specimens stored at -20 ^{o}C were relatively constant while urine stored at ≥25 ^{o}C achieved pH results >9. None of the storage conditions produced a urine specimen with a pH >9.5. After the two week incubation, urine specific gravity was stable at all the tested storage temperatures while urine creatinine was stable at –20, 4, and 25 ^{o}C and unstable at elevated temperatures. The inclusions of microorganisms (bacteria or yeast), protein, and/or glucose at pathological levels did not have any significant effect on urine pH values when compared to the neat urine, excluding the protective effect of glucose at 93 ^{o}C. Degradation of non-protein nitrogenous urine analytes, such as creatinine, urea, and uric acid, is most likely responsible for the in vitro increases in pH observed.

Conclusions: Specimen validity testing criteria are important to identify those urine specimens which may have decreased drugs of abuse recoveries because of pH changes, either caused by environmental storage conditions or attempted adulteration. The effect of increased time and temperature alone were sufficient to increase urine pH to values >9.0. Microbial contamination, proteinuria, and glucosuria did not contribute to the increasing urine pH values found with storage at increased temperatures. The results of this study are important supplemental information for the MRO and should be considered when reviewing invalid specimen results in the pH 9.0-9.5 range for alternative, non-medical explanations.

Keywords: **pH Validity Testing, Workplace Drug Testing, Urine Drug Testing**

*******2007 ERA RECIPIENT*******

P1 Two-Dimensional GC/EI-MS with Cryofocusing for Sensitive and Specific Quantification of MDMA, MDA, HMMA, HMA, and MDEA in Human Plasma

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To our knowledge, this is the first analytical methodology devised for simultaneous quantification of 3,4-methylenedioxymethamphetamine (MDMA or ecstasy), 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMMA), 4-hydroxy-3-methoxyamphetamine (HMA) and 3,4-methylenedioxyethylamphetamine (MDEA) in human plasma. This is one of the few forensic toxicology methods reported to date that utilizes two-dimensional gas chromatography/electron impact-mass spectrometry (2d-GC/EI-MS). Internal standard solution containing MDMA-d5, MDA-d5, MDEA-d6 and pholedrine was added to 1 mL of plasma. Following acid hydrolysis at 100ºC, pH was adjusted to 6 using 10 M sodium hydroxide and phosphate buffer (pH 6). Hydrolyzed samples were applied to conditioned StyreScreen DBX extraction columns and washed with H_2O, 0.1 M acetic acid and methanol using light positive pressure. Columns were dried and analytes eluted with ethyl acetate/ isopropanol/ ammonium hydroxide (90/6/4, v/v/v). Following addition of methanolic HCl, samples were dried under nitrogen at 35ºC, reconstituted with 0.05 M triethylamine in heptane and derivatized using heptafluorobutyric acid anhydride. Samples were back extracted into TRIS buffer (pH 7.4), vortexed, centrifuged and the top organic layer transferred into autosampler vials. 2d-GC separation of analyte fluoroacyl derivatives was accomplished with two capillary columns in series coupled via a pneumatic Deans switch system. Individual analytes eluting from the primary (DB-1MS) column were selected and cryofocused prior to resolution on the secondary (ZB-50) column. Detection and quantification were accomplished with a single quadrupole mass spectrometer operated in electron impact-selected ion monitoring mode. Method validation included determination of linearity, limits of detection (LOD) and quantification (LOQ), accuracy, precision, specificity, recovery, carryover, dilution integrity and stability. The LOQ was 2.5 ng/mL for all analytes except MDA, which had a 1 ng/mL LOQ. Using 1/x weighting, calibration curves (n = 4) for MDA were linear from 1-100 ng/mL, HMA from 2.5-100 ng/mL and MDMA, HMMA and MDEA from 2.5-400 ng/mL. Coefficients of determination for all analytes were $\geq$ 0.997. Accuracy was ± 20% of target concentration and between and within day precision were $\geq$ 90.3%. 69 potential exogenous interferents were evaluated by spiking 1000 ng/mL of interferent into low concentration quality control (QC) samples; all spiked samples quantified within ± 20% of mean QC concentration. Extraction efficiency was constant across concentration and ranged from 80-90% for all analytes. Dilution studies indicated that accurate measurement of analytes can be made by dilution of concentrated specimens with 0.1 M pH 6.0 phosphate buffer. Stability of all analytes except HMA was 96.6-104.0% following 12-h ambient, 48-h refrigerated, and freeze/thaw (3 cycles) challenges; HMA showed variability at lower concentrations. Carryover was not evident following injection of a 1000 ng/mL sample. This method has been thoroughly evaluated and found to be a rugged, sensitive and specific method appropriate for the concurrent quantification of MDMA, MDA, HMMA, HMA, and MDEA in human plasma.

Keywords: **MDMA, Plasma, GC/MS**

*******2007 ERA RECIPIENT*******

P2 Propofol in Pig Breath and Plasma

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The analysis of exhaled breath is a non-invasive alternative with many potential biomedical applications. A common application of breath analysis is monitoring breath concentrations of ethanol for the enforcement of traffic safety. The ability to detect ethanol in breath is valuable because breath concentrations correlate with blood concentrations, as well as physiological and behavioral patterns. A relationship between blood and breath potentially exists not only for ethanol, but many other compounds including propofol.

Propofol is a popular anesthetic agent used in a variety of clinical settings. Propofol is administered intravenously. It is highly lipophilic and its dosage cannot be titrated like traditional gaseous anesthetics. Preliminary studies have shown that propofol can be detected in exhaled breath; therefore, breath analysis may be an excellent non-invasive technique for monitoring patients undergoing clinical procedures.

Preliminary studies were conducted with pigs to investigate the relationship between breath and plasma concentrations of propofol. With approval from the University of Florida Institutional Animal Care and Use Committee, the pigs were anesthetized and intubated under veterinary anesthetic agents such as ketamine, telazol, and xylazine. Anesthesia was maintained with isoflurane, which supplemented propofol administration. Catheters were placed into one artery and two veins in order to collect arterial and venous plasma specimens at specific time intervals as well as provide an additional venous line for propofol infusion separate from the collection site. Propofol was administered with target infusion rates between 50 and 300 µg/kg/min. Breath samples were collected onto glass traps containing XAD-2 resin (Orbo 605, Sigma-Aldrich, Inc., St. Louis, MO) at timed intervals coordinated with the collection of the plasma specimens. Additional breath specimens were also taken to monitor the concentrations of propofol in breath throughout the experiment.

Propofol, with deuterated propofol (d_{18}-propofol, Cerilliant, Round Rock, TX) as the internal standard, was extracted by solid-phase extraction (SPE) and quantitated by gas chromatography-mass spectrometry (GC-MS). Propofol in breath was isolated from the resin by solvent desorption. Propofol was isolated from the acidified plasma specimens using Styre Screen C18 columns (UCT, Bristol, PA). Extracts of the breath and plasma specimens were reconstituted in methanol and analyzed by GC-MS. These methods were validated using quality control specimens prepared in corresponding blank matrices. Plasma concentrations of propofol typically ranged between 0.10 and 6.2 µg/mL. Exhaled breath concentrations were less than 3.2×10^{-4} µg/mL, which resulted in arterial plasma-to-exhaled breath propofol ratios ranging between 1.02×10^{4} and 12.1×10^{4}. These findings suggest differences in the plasma-to-breath ratio depending on the pharmacokinetic state of the pig. Future work is required to continue understanding the relationship between plasma and breath concentrations of propofol.

By optimizing the collection of breath, as well as developing protocols for the isolation and quantitation of drugs in the relevant matrices, this project serves as a model for many other clinically-relevant compounds.

Keywords: **Propofol, Breath, GC-MS**

*******2007 ERA RECIPIENT*******

P3 Rapid Screening of Six Postmortem Urine Samples for Fentanyl and Norfentanyl by Direct-Injection Mass Spectrometry

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It is the aim of this study to develop a rapid method to screen postmortem forensic urine samples for fentanyl and its major hepatic metabolite, norfentanyl. Minimal sample preparation consisted of a five minute centrifugation at 13,000 rpm to remove particulate matter. This was followed by a quick off-line solid-phase extraction procedure (C18 Sep-Pak®, Waters Corp, Milford, MA) to remove the majority of salts, which are largely responsible for ion suppression from urinary matrix effects. Samples were spiked with deuterated internal standards (D_5-fentanyl and D_5-norfentanyl, Cerilliant Corp, Round Rock, TX) at a final concentration of 10 ng/mL, which is the recommended detection limit set by the SOFT Drug-Facilitated Sexual Assault Committee. Stable-labeled internal standards were used to compensate for instrument variation in signal, analyte recovery during sample preparation, and ion suppression. Structural information for fentanyl and norfentanyl were collected using three mass spectrometer instruments, each equipped with electrospray ionization (ESI) and operated in the positive ion mode. A full toxicological blood analysis was previously performed on the six drug-overdose cases involving fentanyl using LC-MS by the Office of the Chief Medical Examiner of West Virginia or NMS Laboratories (Willow Grove, PA). Urine samples from the six cases were not previously analyzed and thus sent to our lab by the Office of the Chief Medical Examiner of West Virginia for detection of fentanyl and norfentanyl. Fentanyl was found in each of the six overdose cases by the appearance of the MS/MS daughter ion on both an ion trap and a triple quadrupole mass spectrometer resulting from the fragmentation pathway of fentanyl (*m/z* 337→188). Norfentanyl was detected in all six cases by the appearance of the MH^+ ion, *m/z* 233, with a single quadrupole mass spectrometer and confirmed in an ion trap mass spectrometer by the appearance of its MS/MS product ion from the transition *m/z* 233→84. Quantitative estimates of analyte concentrations were assessed based on the ratios of analyte:internal standard. Ratios were calibrated with a seven-point standard curve (r^2=0.999). Ion suppression, as determined by the comparison of ion intensities from spiked samples in water with postmortem urine from the cases, ranged from 18% to 98% in three ESI sources. The use of deuterated internal standards obviates extensive sample preparation and chromatography because ratios of analyte:internal standard remain constant in the presence of extensive matrix effects. This mass spectrometric method provided sufficient sensitivity and selectivity for the rapid identification of fentanyl and norfentanyl in urine at levels ≥ 10 ng/mL without prior analyte purification by chromatography, and with a total analysis time of less than one hour.

Keywords: **Fentanyl, Mass Spectrometry, Stable Isotopes, Postmortem**

*******YSMA AWARD RECIPIENT*******

P4 Buprenorphine Concentrations in Post Mortem Whole Blood and Urine from Suspected Overdose Cases

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Buprenorphine is a partial mu-opioid receptor agonist, and is used for the treatment of moderate to severe chronic pain. Since buprenorphine has a fairly slow onset as compared to e.g. heroin, it has become popular as a safe drug in opiate substitution therapy, and considered to have a low abuse potential. Some years after its introduction on the Swedish market, illegal use of buprenorphine started, and several deaths involving buprenorphine has been documented in Sweden. We explored the femoral blood and urine levels, and evaluated the role of buprenorphine in these deaths in the light of the total toxicological findings.

Buprenorphine in urine was analyzed accordingly to a previously published procedure[1]. The method for blood was developed and validated prior to this study and is briefly described as follows. Two grams of post-mortem blood were precipitated using acetone. The acetone phase was then transferred to a new tube, evaporated and the residue diluted with 3 mL of phosphate buffer (pH 6.1). Then SPE was performed as for the urine samples[1]. The LC/MS/MS analysis was performed on a Perkin Elmer 200 chromatography system and an API 2000 triple quadrupole instrument equipped with an electrospray interface operating in the multiple reaction monitoring (MRM) mode. Two transitions were monitored for each analyte. Linear calibration curves were established between 0.2 and 5 ng/g and between 0.5 and 5 ng/g for buprenorphine and norbuprenorphine respectively.

The femoral blood concentrations of buprenorphine and norpbuprenorphine varied substantially. The means ± SD of the buprenorphine concentrations in postmortem femoral blood (n=32) were 3.1 ± 4.7 with a median of 1.1 ng/g. In most cases, several other CNS depressant drugs were present that together with buprenorphine contributed to the death. Interestingly, the concentrations in 11 obvious intoxication cases, in which buprenorphine was indicated as a major or contributory player, the concentrations were not different from either the mean or median of the whole postmortem group, and additionally, in four postmortem cases, where intoxication was definitely ruled out, the mean concentration was higher. Hence it seemed impossible to define an exact level that is fatal. Instead the results suggest that other factors may determine when an overdose involving buprenorphine will have a fatal outcome, and one of the most suspected candidates is abstinence. Therefore, we investigated the proportions of the parent drug and the metabolite in both blood and urine. In all cases, the mean urine concentrations were 125 ng/mL and 214 ng/mL for buprenorphine and norbuprenorphine, whereas in the 11 cases the mean values were 89 ng/mL and 27 ng/mL. In 9 of the 11 cases classified as buprenorphine deaths the blood buprenorphine concentration was higher than that of norbuprenorphine suggesting that their presence in blood represented an acute intake and in 8 cases this was combined with absence or low concentrations of norbuprenorphine in urine supporting a relatively long period of abstinence prior to the last dose.

We concluded that recent abstinence was more common in the deaths where buprenorphine was considered the main cause of death.

1. Kronstrand R, Seldén T, and Josefsson M. Analysis of buprenorphine, norbuprenorphine, and their glucuronides in urine by liquid chromatography-mass spectrometry *J Anal Toxicol* 2003, vol. 27, pp. 464-470

Keywords: **Buprenorphine, Postmortem, Intoxication**

P5 Effect of Variation in Site and Time on the Level of Ethanol and Methanol in Postmortem Blood Samples

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It is well recognized that postmortem (PM) drug levels in blood may be unstable as a consequence of redistribution artifact. When measuring drug concentrations after death, it is important to consider the phenomenon of PM drug redistribution. PM drug concentrations may not be a true reflection of the antemortem one and as a result, wrong conclusions could be made about the cause of death. There are few published studies for most drugs and poisons to show the important differences in their PM concentrations in blood and tissues according to choice of sampling site, sampling time, handling of samples including containers, preservation and documentation and type of laboratory investigation carried out on PM samples.

The present work was carried out to evaluate experimentally in rabbits PM behavior of ethyl and methyl alcohol in relation to their concentration in different blood sampling sites at different time intervals. Furthermore to assess the effect of site blood sampling on the level of ethyl alcohol and methyl alcohol at time of autopsy in human cadavers and compare it with the results from rabbit experiments.

The study was conducted on ninety male rabbits as experimental animals, and the human cadavers that were positive on screening to ethanol (n=4) and methanol (n=3) during the period of the study. Rabbits were divided into three groups (30 rabbits each), two groups for each drug, which were given the LD50 of the drug. Ninety minutes after dosing all rabbits were sacrificed by CO_2 inhalation. Blood samples were drawn from right and left sides of the heart and femoral vein from each group of rabbits, immediately, twelve hours and twenty-four hours after death. As regards human cadavers, blood samples (5mL) were drawn from right and left sides of the heart and femoral vein at time of autopsy. Experimental and human blood sample extracts were analyzed by gas chromatography.

The study showed that ethanol was detected in the control group after 12h PM. The highest mean value recorded was 681 µg/ml in 24h PM Rt. cardiac. No significant changes could be detected in immediate PM blood concentration for ethanol and methanol from different sampling sites. The study also revealed that PM blood concentration for ethanol and methanol increased over time for different sampling sites. Where up to 24h PM femoral (peripheral) blood drug concentrations were the closest to the immediate PM values, followed by Rt. cardiac then Lt. cardiac blood. It was noticed also that up to 12h PM femoral (peripheral) blood methanol concentration could be used as a reliable indicator for the immediate PM values.

Keywords: **Postmortem, Ethanol, Methanol**

P6 Postmortem Ethanol Testing Procedures Available to Accident Investigators

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This paper intends to provide investigators with information useful in determining the presence of postmortem ethanol in fatal accidents and an example of a case history of an automobile accident that involved postmortem alcohol formation. Specimens collected 60 hours after death at autopsy in this case revealed 0.07% ethanol in urine and 0.08% ethanol in both heart and cavity blood. The ethanol positive fatal case initially was reported as being from ingestion, but was ultimately determined to be from postmortem ethanol production using the ratio of two serotonin metabolites found in urine. The serotonin metabolite ratio was determined using LC/MS^n. Ethanol consumption causes a significant change in the ratio of 2 serotonin metabolites, 5-Hydroxyindole-3-Acetic Acid (5-HIAA), and 5-Hydroxytryptophol (5-HTOL). Published research has shown that a 5-HTOL(pg/mL)/5-HIAA(ng/mL) ratio at or above 15 indicates ethanol consumption in the last 8-12 hours. In the case reported here the ratio was less than 1 indicating no recent ethanol ingestion. This case involved a transportation accident that could have resulted in additional hardships for the victim's family through loss of compensation and reputation.

Keywords: **Ethanol, Postmortem, Accident Investigation**

P7 Age-Related Differences in Blood Alcohol Concentration in Acute Alcohol Intoxication Fatalities

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The purpose of this study was to evaluate blood alcohol concentrations from acute alcohol intoxication fatalities to determine if younger individuals are more susceptible to acute alcohol intoxication at lower blood alcohol concentrations. This retrospective study was undertaken to examine the alcohol concentrations in acute alcohol fatalities investigated by the Office of the Chief Medical Examiner of the State of Maryland between January 1, 1990 and May 1, 2007. Case selection criteria included a cause of death of "Acute Alcohol Intoxication" and the absence of other toxicological or pathological findings that contributed to the death. During this period, 148 cases that met these criteria were investigated. The demographic makeup and blood alcohol concentrations of these cases were:

Age (yrs)	# Male	# Female	Mean (range) BAC (% ,w/v)	% with BAC <0.40%(w/v)
Under 20	4	0	0.31 (0.24 – 0.34)	100
20 - 29	20	2	0.35 (0.18 – 0.50)	82
30 – 39	41	5	0.41 (0.22 – 0.62)	50
40 – 49	37	10	0.41 (0.14 – 0.74)	47
50 – 59	20	6	0.39 (0.19 – 0.61)	58
60 - 69	2	1	0.36 (0.27 – 0.41)	67

A blood alcohol concentration of 0.40% (w/v) or above is generally accepted as an acutely fatal blood alcohol concentration. In the cases examined in this study, 84 (57%) had a heart or subclavian blood alcohol concentration less than 0.40% (w/v). Acute alcohol intoxications associated with a BAC <0.40 were seen more frequently in decedents under the age of 30. In addition, postmortem blood alcohol concentrations as low as 0.14 %(w/v) were associated with acute alcohol intoxication as a cause of death.

Keywords: **Acute Alcohol Intoxication, Postmortem, Alcohol**

P8 Validation of EMIT Screening for Oxycodone in Postmortem Vitreous Humor

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Laboratory guidelines put forth by a committee of SOFT/AAFS state that it is good practice in postmortem toxicology to confirm the identity of an analyte in a second sample. Often, an acceptable blood or urine sample is not available at autopsy. Vitreous humor has long been known to be a preferred sample for analysis of alcohol and digoxin. This fluid is essentially a sterile saline solution with proteins present at low concentrations. The goal of this study was to validate the use of postmortem vitreous humor as an alternative matrix for oxycodone screening and to compare vitreous humor results to those obtained from blood precipitates.

Oxycodone is a semi-synthetic opiate used to treat moderate to severe pain. Since the approval of the controlled-release form in 1995, many areas in the US have seen a dramatic increase in the number of deaths attributed to oxycodone. In order to determine the presence of oxycodone or another drug of abuse, forensic laboratories typically use immunoassay testing of urine or blood to quickly and efficiently screen postmortem samples.

A pool of opiate negative vitreous fluid was prepared from cases in which blood precipitates were negative on both Opiate and Oxycodone DRI (EMIT®) assays. Aliquots were spiked with oxycodone at concentrations ranging from 5-4000 ng/mL. These were then analyzed on a Microgenics MGC 240 instrument with DRI Oxycodone kits (EMIT®) according to the manufacturer's specifications for urine samples. After determining an LOD of 25 ng/mL, the assay was evaluated using vitreous humor standards over the range of 25 to 4000 ng/mL and found to be linear from 25 to 500 ng/mL. Interday precision was 5.5 to 11.8 % for standards over the range of 50 to 4000 ng/mL. The intraday precision was evaluated at 50, 200, and 500 ng/mL and ranged from 2.4 to 4.1 %.

Vitreous humor from 61 cases in which blood precipitates screened positive for oxycodone was analyzed using the validated immunoassay curve. The plot of the blood precipitate immunoassay versus the vitreous humor immunoassay resulted in a correlation coefficient of $r = 0.85$ ($p < 0.01$). All vitreous humor samples that were positive for oxycodone and available in a volume of 0.5 mL or more were confirmed and quantitated by GC/MS. The oxycodone screening of vitreous humor yielded positive results in all samples where oxycodone was present in the blood. No false positives were obtained on vitreous humor screening of samples from cases in which blood was negative for oxycodone ($n = 14$). In conclusion, enzyme immunoassay screening of vitreous humor for oxycodone may serve as a reliable alternative to the screening of blood or urine.

Keywords: **Vitreous Humor, Oxycodone, Immunoassay, Postmortem**

P9 Comparison of Oxycodone Concentrations in Vitreous Humor and Postmortem Blood

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Oxycodone was the third most commonly encountered opioid drug in fatal overdose cases in West Virginia in 2006, led only by methadone and hydrocodone. Oxycodone is a commonly detected drug in many postmortem toxicology laboratories and its quantitation in postmortem samples is of utmost importance. In death investigations where postmortem blood samples are not available or are of limited quality or quantity, vitreous humor may prove to be a useful alternative specimen for the detection and quantitation of oxycodone. The purpose of the present study was to assess the extent to which vitreous humor oxycodone concentrations correlate with concentrations in blood.

The use of vitreous humor as an alternative specimen to whole blood for confirmation and quantitation of oxycodone in postmortem specimens was investigated in 22 cases of fatal oxycodone overdose evaluated by the Office of the Chief Medical Examiner. Analysis was performed by GC/MS using D6-oxycodone as an internal standard. Calibrators for whole blood samples consisted of negative serum spiked with oxycodone at concentrations of 5, 10, 50, 500, 1000, and 2000 ng/mL. Calibrators for vitreous humor samples consisted of negative vitreous humor spiked with oxycodone at concentrations of 10, 50, and 500 ng/mL. The vitreous humor assay was found to be linear over this range with a limit of detection of 7.5 ng/mL and a limit of quantitation of 10 ng/mL. Inter-day precision, measured as %RSD, was calculated at 1.9% and intra-day precision at 1.0% for vitreous humor spiked at a concentration of 100 ng/mL.

The possibility of a correlation between oxycodone concentrations in whole blood and vitreous humor was investigated in fatal oxycodone overdose cases. Blood samples from these cases, the majority of which were collected from the subclavian vein, screened positive for oxycodone by immunoassay and were confirmed by GC/MS. Oxycodone was extracted from blood and vitreous humor using solid-phase extraction and samples were analyzed by GC/MS. Oxycodone concentrations in blood ranged from 100 ng/mL to 1,450 ng/mL and concentrations in vitreous humor ranged from 40 ng/mL to 680 ng/mL. Vitreous-to-blood ratios ranged from 0.30 to 1.30, with a mean ratio of 0.93 ± 0.28. In 19 of the 22 cases, oxycodone blood and vitreous humor concentrations were correlated ($r = 0.79$). Possible explanations for the three cases which were not correlated include drug redistribution during the postmortem interval and delayed partitioning of oxycodone into vitreous humor in the instance of rapidly increasing blood concentration.

Keywords: **Oxycodone, Vitreous Humor, Postmortem**

P10 Detection of Cocaine in Postmortem Oral Swab Samples

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One of the most active areas of forensic toxicology research is the investigation of hair, sweat and oral fluid as alternative matrices for drug testing. However, the use of oral swab samples obtained postmortem has not been as thoroughly addressed. Forensic laboratories commonly use enzyme immunoassay of blood or urine to screen for evidence of drug use, but these samples are not always readily available at autopsy. Therefore, the goal of this study was to evaluate oral swabs collected postmortem as alternative samples for the detection of cocaine.

Validation studies were performed on standard laboratory cotton tipped swabs spiked with known concentrations of cocaine dissolved in 100 uL of blank saliva collected from non-cocaine using donors. A simple method was used in which swabs were eluted into 1.0 mL of methanol, centrifuged, decanted, and dried under nitrogen. The residue was then reconstituted in methanol and 2.0 uL were injected onto the GC-MS for analysis in full scan mode. A deuterated internal standard (Cocaine-D3) was added before centrifugation to allow for quantitation. A calibration curve was constructed at cocaine concentrations of 25, 50, 200, 500 and 1000 ng/mL. Controls spiked at 65, 100 and 400 ng/mL were included to demonstrate accuracy and precision. The method was evaluated over the range of 7.5 – 1000 ng/mL, with a limit of detection of 10 ng/mL and a limit of quantitation of 25 ng/mL. Interday samples run in triplicate resulted in recovery of 99.3 ± 5.4%. The intraday samples, also run in triplicate, resulted in an average recovery of 105.9 ± 6.6%.

Oral swabs were collected from 15 postmortem cases in which drug overdose was the suspected cause of death and the presence of cocaine and/or benzoylecgonine was confirmed by GC-MS analysis of peripheral blood. The average amount of material collected on the swabs was 85 mg ± 48 mg. Cocaine was detected in 12 oral swabs with the amount of cocaine on the swab ranging from < 25 ng/swab to > 1000 ng/swab. Additionally, cocaine was detected in oral swabs from two cases in which only the principle cocaine metabolite, benzoylecgonine, was detected in blood. In conclusion, oral swab samples obtained prior to autopsy may serve as useful specimens for identifying cocaine use.

Keywords: **Oral Swabs, Cocaine, Postmortem**

P11 Determination of Cocaine, Cocaethylene and Benzolyecgonine in Blood and Brain by LC-MS/MS

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The objective of these analyses was to validate a liquid chromatographic-mass spectrometric-mass spectrometric (LC-MS/MS) analysis of cocaine (COC), cocaethylene (COCA) and benzoylecgonine (BE) for low level determinations in post-mortem specimens.

The method employs LC-MS/MS with positive electrospray ionization. Calibrators and positive controls were prepared by adding known amounts of COC, BE and COCA and their deuterated analogs (COC-D_3, BE-D_3 and COCA-D_3) into 0.5 ml of whole blood. Negative controls were prepared to contain only the internal standards. Brain specimens were homogenized in deionized water (1:4). Analytes were extracted onto United Chemical Technologies Clean Screen solid phase columns. Column eluates were chromatographed through a gradient elution using H_2O: CH_3CN + 0.1% CHOOH (0.25 mL/min) on a Varian Pursuit column (50mm, 2.0 mm i.d., 3 μ, C18).

Analyses were conducted with a Varian 1200 triple quadrupole instrument operating in the multiple reaction monitoring (MRM) mode under the following conditions: capillary volts, 63 V; drying gas temp 400 ^oC; drying gas pressure, 24 psi; nebulizing gas, 55 psi; ESI needle voltage, 1500 V; shield voltage, 800V; and collision pressure, 1.5 mTorr. Precursor and product ions were (quant. ions underlined): COC: 304.2 → <u>82</u>, 105, 150; BE: 290.2→ <u>168</u>, 105, 119; COCA: 318.3→ <u>196</u>, 150, 82; COC-D_3: 307.2→ <u>185</u>, 105, 85; BE-D_3: 293.2 →<u>171</u>, 105, 85; COCA-D_3: 321.2 →<u>199</u>, 153, 85.

Linearity (r^2 >0.99) was demonstrated from 0.5 to 200 ng/mL for each analyte. Assay accuracy and precision are shown in Table 1.

Table 1. Assay accuracy and precision (n = 5)

Analyte	COC		BE		COCA	
Target (ng/ml)	2	100	2	100	2	100
MEAN	1.99	98.82	2.05	95.60	2.03	108.60
Std Dev	0.34	4.06	0.15	6.73	0.11	2.61
CV %	17.2	4.1	7.3	7.0	5.1	2.4

Data in Table 2 demonstrate the utility of LC-MS/MS towards determination of small amounts of COC and its metabolites in forensic specimens.

Table 2. COC, BE and COCA in blood and brain from three Medical Examiner cases

Case #	COC		BE		COCA	
	Blood (mg/L)	Brain (mg/kg)	Blood (mg/L)	Brain (mg/kg)	Blood (mg/L)	Brain (mg/kg)
1	nd	0.004	0.69	0.69	nd	nd
2	0.004	0.01	0.11	0.09	0.008	0.008
3	0.001	0.12	0.04	0.03	nd	< 0.004

Keywords: Cocaine, LC/MS/MS, Postmortem

P12 The Distribution of Sertraline in Postmortem Fluids and Tissues

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Due to the violent collisions often associated with aircraft accidents, victims' bodies may be fragmented and/or incinerated. In many cases, the search for remains results in only small fragments of various tissues available for toxicological analysis. In fact, the Federal Aviation Administration's Forensic Toxicology and Accident Research Laboratory receives blood specimens in only approximately 70% of fatal aviation accidents. However, therapeutic and toxic concentrations for most drugs are reported in the scientific literature for blood and plasma only. Sertraline, (1*S*)-*cis*-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine, is a selective serotonin reuptake inhibitor (SSRI) that is sold under the brand name Zoloft® and was introduced in 1991. Sertraline is prescribed for the treatment of depression, anxiety, and obsessive compulsive disorder (OCD). Certain side effects of this medication including, drowsiness, dizziness, abnormal vision, diarrhea, tremor, and headache, could affect pilot performance and become a factor in an aviation accident. Our laboratory has determined distribution coefficients for sertraline and its desmethyl metabolite norsertraline in various postmortem tissues and fluids from 10 cases previously found positive for this drug. If available, 11 specimen types were analyzed for each case including: blood, urine, vitreous humor, bile, liver, kidney, skeletal muscle, lung, spleen, heart muscle, and brain. Specimens were extracted using solid-phase extraction and analyzed by GC/MS. Blood sertraline concentrations in these 10 cases ranged from 28 to 3002 ng/mL. Nine of these 10 cases had blood concentrations that would normally be expected for an individual taking a prescribed amount of sertraline. Distribution data for this compound and its metabolite will be presented.

Table 1: Sertraline distribution coefficients* determined for each specimen type examined.

	Urine	Vitreous Humor	Bile	Liver	Kidney	Skeletal Muscle	Brain	Spleen	Lung	Heart Muscle
n	5	4	6	9	9	7	8	8	8	9
Mean	0.44	0.014	52	82	7.7	2.8	23	26	56	11
Range	0.018-1.15	0.0013-0.031	16-78	12-186	3.7-18	0.9-5.8	10-58	7-48	11-131	1.6-32

* distribution coefficients determined by dividing specimen concentrations by the blood value obtained from each case.

Keywords: **Sertraline, Tissue Distribution, Postmortem**

P13 Antiemetics with Concomitant Sedative use in Civil Aviation Pilot Fatalities from 2000 to 2006

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Many drugs commonly used for the treatment of various ailments can be dangerous when used in combination. Antiemetics and sedatives are two drug classes that contain compounds that may have harmful side effects when mixed. An antiemetic medication such as chlorpheniramine can dramatically increase the negative side effects of numerous drugs in the sedative class. This phenomenon is especially dangerous for pilots that are in control of an aircraft. Although many of these compounds are considered disqualifying and not allowed by the FAA their use does occur in the pilot community. Many of the pilots that use these drugs are unaware of the danger that may arise when compounds from these two drug classes are taken together. Our laboratory was interested in evaluating the circumstances surrounding accidents in which the pilot was found positive for drugs from each of these two classes. Epidemiological, toxicological and aeromedical findings from pilots involved in such accidents were collected for a 7-year period, 2000 - 2006. Case histories, accident information, and the probable cause of the aviation accidents were obtained from the National Transportation Safety Board (NTSB). Toxicological information was obtained from the Civil Aerospace Medical Institute's (CAMI's) Forensic Toxicology Research Laboratory. There were 2184 fatal aviation accidents over this time period. Of these accidents, 26 were found positive for compounds from both the antiemetic and the sedative drug classes. All 26 aircrafts were operated under 14 CFR Part 91 as general aviation. All pilots involved in these accidents were male. Twenty-one of the 26 pilots tested positive for a disqualifying substance that may have affected their ability to control the aircraft.

Keywords: **Antiemetics, Sedatives, Aviation**

P14 A Case of Acute Arsenic Poisoning and Hundred-Year Review of Arsenic Tissue Distribution in Acute or Sub-acute Poisoning

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During the 19th and first part of the 20th century acute arsenic poisoning was a common occurrence and as such, forensic toxicologists were well acquainted with not only the physical signs, symptoms and pathological findings in acute arsenic poisoning; but, also the expected distribution of arsenic throughout the body tissues and fluids. As acute arsenic poisoning is relatively rare today, we present a case of acute arsenic poisoning and a summation of a review covering the last hundred years of the published literature of the tissue distribution of arsenic in cases of acute and sub-acute poisoning. The presented case involved a 46 year-old, white male who mistakenly drank 13 –16 fluid ounces of a 4% solution of sodium arsenate. Shortly after consumption, he became violently ill, presenting at a local emergency department with violent vomiting, extreme hypotension and a weak tready pulse. Despite emergency treatment, he died of multiorgan failure within 3 hours of admission. Gross pathological findings included irregular ulcers of the distal esophagus, fatty change in the liver, bilateral pleural effusions and spleenomegaly. Microscopic examination revealed renal tubular necrosis and hepatic fatty change. Arsenic was determined by colorimeteric analysis of autopsy specimens using arsine generation and trapping with silver diethyldithiocarbamate. Postmortem toxicological findings in mg/L or mg/Kg were: blood, 6.7; brain, 5.2; kidney, 52; liver, 94; lung, 32; pancreas, 38; spleen, 43; and 84 mg of total arsenic were recovered in the stomach contents. This arsenic tissue distribution is consistent with distribution findings of over 100 cases of acute poisoning from ingestion of arsenic reported in the literature over the past hundred years. Numerous factors including the particular arsenic compound ingested, survival time and emergency treatment with antidote administration may result in wide variations in the absolute concentrations of arsenic found in the body tissues. However, in cases of acute or sub-acute ingestion of arsenic, liver and kidney contain the highest concentrations of arsenic. Large concentrations of arsenic, typically fifty to a hundred times normal concentrations, are found in all body fluids or organs; blood, brain, heart, lung, pancreas, skeletal muscle and spleen. The stomach and intestinal specimens, as well as their contents, usually contain massive amounts of arsenic even after intensive treatment. This case and the summation of over 100 fatal acute poisonings provide information helpful to toxicologists when evaluating arsenic tissue distribution in cases of acute and sub-acute arsenic poisonings.

Keywords: **Arsenic, Fatal Poisoning, Tissue Distribution**

P15 Distribution of Propofol in Two Cases of Self-Administration Deaths

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Propofol (2,6-diisopropylphenol, Diprivan®) is a drug with sedative-hypnotic properties that is used for the induction and maintenance of anesthesia. Although there are a number of reported deaths associated with propofol use, there is limited information on the distribution of the drug into tissue. The purpose of this study is to present quantitative data on the distribution of propofol in two postmortem cases involving propofol intoxication.

Case 1 involved a 44 year old white male who was employed as a nurse anesthetist. He committed suicide by self administering propofol mixed in a 50 mL saline bag. No alcohol or drugs other than propofol were detected. Case 2 involved a 24 year old white male who reportedly self-administered propofol, fell unconscious and died. A visual inspection of the body detailed injection marks on his feet and arms, suggesting previous self-administration. In addition to propofol, zolpidem was detected in the blood at 0.11 mg/L. Zolpidem and diphenhydramine were present in the urine.

Propofol was quantitated in the biological specimens by gas chromatography/ mass spectrometry (GC/MS). An Agilent 6890 GC with a 5973N mass selective detector operating in selected ion monitoring (SIM) mode was used for the identification and quantitation of propofol. A DB-5MS column (20 m x 0.18-mm i.d., 0.18 μm film thickness) was used for analytical separation. Three ions were chosen for identification of propofol, m/z 163*, 178, and 117 (* denotes the quantitation ion). Methylphenidate was used as the internal standard; m/z 84 was monitored. The specimens were made basic with the addition of 2 mL sodium borate buffer (pH 9.4) and extracted with 500 μL of chloroform/ethyl acetate (7:3). After mixing and centrifugation, 1 μL of the organic layer was injected into the GC/MS. The following results were obtained:

Propofol Distribution (mg/L or mg/kg)

Specimen	Case 1	Case 2
Heart blood	4.43	1.04
Brain	16.90	2.93
Kidney	5.49	1.75
Liver	11.60	4.10
Lung	7.29	1.45
Spleen	4.98	1.14

The blood concentrations in both cases were within the therapeutic range of propofol (1.0 – 4.0 mg/L). However, administration of propofol is usually performed in a hospital setting where respiratory assistance can be provided as required. Without medical assistance, the blood concentrations observed can explain death in these two cases.

Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Defense or of the Army, Navy or Air Force.

Keywords: **Propofol, Postmortem, Forensic Toxicology**

P16 Case Report: Death due to Dichloromethane Toxicity

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Dichloromethane (DCM) is an organic solvent whose volatility and ability to dissolve other organic compounds makes it an ideal component in many chemical processes. DCM is commonly found in: paint strippers, degreasers, aerosol spray propellant, and as an agent for blowing foams. It is widely known that the inhalation of DCM can lead to severe intoxication affecting the central nervous and cardiovascular systems. Death may occur from narcosis and respiratory depression.

This report documents the DCM-related death of a 23 year-old Hispanic male (5'8"; 237 lbs) found unresponsive lying face down on a bathroom floor and pronounced dead at the scene. The decedent was employed as a commercial cleaner and was on the job when he was found. He had last been known alive about 6½ hours earlier when he entered the bathroom and closed the door. There were no open windows and only a small overhead fan for ventilation. In addition, the decedent was not wearing a protective mask or respirator. The decedent had been using several chemicals in the bathroom, including Benco #B4®, an industrial paint remover. This product contains approximately 70 to 85% DCM.

An autopsy revealed a ½" superficial abrasion on the nasal bridge, while no other evidence was observed. The internal examination revealed biventricular dilatation (heart weight = 490g) and biventricular myocyte hypertrophy, in addition to pulmonary congestion with moderate amounts of bloody fluid. No ethanol was detected in the blood; no therapeutic or abused drugs were detected in the urine. The carboxyhemoglobin saturation level was < 10%. DCM was detected in the blood by headspace gas chromatography and confirmed by headspace gas chromatography-mass spectrometry (HS GC-MS). Submitted specimens were quantitated by HS GC-MS. The standard curve was linear from the range of 0.625-80 mg/L over a 7-point curve ($r = .9999$ and $y = 0.2013x-0.0188$). The cause of death was ruled DCM toxicity complicating dilated cardiomegaly and the manner of death was accident.

Specimen	DCM (mg/L or mg/kg)
Kidney	3.8
Liver	46.8
Bile	1.0
Peripheral Blood	31.8
Heart Blood	23.0

Keywords: **Dichloromethane, Tissue Distribution, Headspace GC/MS, Postmortem**

P17 Metabolism and Distribution of Strychnine in a Fatal Intoxication

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Strychnine is an alkaloid derived from the seeds of the Strychnos plant (*S. nux-vomica*), which is native to India. Strychnine was used medicinally to treat a range of ailments including gastric problems, circulatory problems and non-ketotic hyperglycinemia. The therapeutic use of strychnine in the United States was suspended in the early 1960s. Other than laboratory and research uses, the only remaining use for strychnine in the US is its restricted use as a pesticide as a below ground bait to control pocket gophers.

Strychnine is rapidly absorbed from the GI tract after oral ingestion. The metabolism of strychnine in man has not been studied, although there have been a few studies examining strychnine metabolism in animals. In humans, strychnine is a potent convulsant, due to a decrease in neuronal inhibition. Strychnine prevents the reuptake of the inhibitory neurotransmitter glycine, resulting in increased neuronal activity and excitability, leading to increased muscular activity. Stimulant activity generally occurs within 15 to 30 minutes following oral strychnine ingestion.

Case History: A 58 year old white male was observed to be seizing and foaming at the mouth in a parking lot. A paramedic unit responded to the scene to find him in full arrest. CPR was initiated and the patient was transported to the hospital, where he remained unconscious and was pronounced dead approximately 15 ½ hours later. Additional investigation indicated that the deceased was a prominent attorney that was running for public office. At the time of his death, he was under investigation for fraud.

Autopsy findings included congested lungs (combined weight = 2560g), bloody fluid in distal and proximal airways and gastritis with bloody gastric contents. In addition, minimal cardiomegaly was present (ht wt = 480g) with biventricular dilatation and tunneling of the left anterior descending artery. Other autopsy findings were unremarkable.

Toxicological analysis identified strychnine, phenytoin, morphine, citalopram and acetaminophen in postmortem specimens from the deceased. Strychnine was detected and quantitated by gas chromatography with nitrogen phosphorus detection and confirmed by gas chromatography with mass spectrometric detection. Strychnine concentrations (mg/L or mg/kg) are displayed below.

Heart Blood	Bile	**Liver**	Kidney	Gastric Conts
7.2	42	24	23	21 mg total

The blood strychnine concentration in this case is within the range of previously reported strychnine fatalities (0.5 to 61 mg/L). In addition, the high concentration of strychnine in the bile is consistent with the extended survival time of the decedent (15 ½ hrs).

In addition to identifying strychnine, GC-MS analysis of the specimens also detected some possible human metabolites of strychnine. It appears that these possible metabolites may consist of dehydrostrychnine, strychnine epoxide and hydroxystrychnine. These compounds were present in the greatest amount in bile, with smaller amounts present in the blood, liver and kidney.

The medical examiner ruled the cause of death as “Strychnine Intoxication” and the manner of death as “Undetermined”.

Keywords: **Strychnine, Postmortem, Metabolism**

P18 Fatal Salicylate Poisoning Secondary to Septicemia as Complications to Atypical Abuse of OxyContin®

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OxyContin® gained approval in 1995 from the Food and Drug Administration as a sustained-release preparation of oxycodone hydrochloride with the belief it exhibited a lower abuse potential than immediate-release forms of the drug because of its slow-release properties. Drug abusers crush and pulverize OxyContin® into a powder, defeating the time-release formulation, so that it may be injected or snorted for the immediate attainment of its euphoric effect. The objective of this presentation is to describe the postmortem toxicological and pathological findings for a decedent succumbing to salicylate poisoning resulting from complications of atypical OxyContin®abuse.

Case history: A 25 year old male was admitted to the hospital with acute toxic reaction and delirium of unknown etiology. He developed respiratory failure and a GI bleed and was placed on a ventilator; however, he experienced rapid deterioration and fatal cardiac arrest within 24 hours of admission. Other symptoms and diagnoses included: Hyperthermia, tachycardia, metabolic acidosis, disseminated intravascular coagulopathy (DIC), and hemorrhagic gastritis. Urine drug screen was positive for opiates and blood cultures grew *Fusobacterium* species. Prior to hospitalization, the decedent complained of an upper respiratory tract infection with sore throat, ringing in the ears and ear pain. He reportedly self-medicated with OTC cold and flu preparations. The decedent's parents indicated to clinicians their son had a history of OxyContin® abuse and experienced very similar symptoms and illness requiring hospitalization two years earlier. Clinicians surmised that bacterial inoculation of normal flora from the oral cavity occurred via mucosal sublingual injection or use of saliva as lubrication for the syringe.

Postmortem toxicology and autopsy findings: Salicylate (566 mg/L), acetaminophen (10 mg/L), doxylamine and dextromethorphan were identified in antemortem hospital blood. Oxycodone was not detected in antemortem hospital serum; however, it was confirmed present, along with oxymorphone, in a urine specimen provided from the hospital. The urine specimen was also remarkable for salicylate (900 mg/L), acetaminophen (6.2 mg/L) and the presence of doxylamine and dextromethorphan. The significant pathological diagnoses at autopsy included multiple petechiae and purpura involving the epicardium, serosal surface of the bowel and white matter of the brain. Hemorrhagic gastritis was also noted. The cause of death is salicylate poisoning and the manner is accident.

Conclusion: Atypical routes of OxyContin® administration in cases of abuse may result in morbid complications. Additionally, ill-fated attempts at self-remedy or excessive OTC medication dosing may contribute to the clinical and toxicological presentation.

Keywords: **OxyContin®, Oxycodone, Septicemia, Salicylate Poisoning**

P19 Incidence of Illicit Fentanyl Usage in Allegheny County (Pittsburgh, PA)

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Since early 2006, Allegheny County (Pittsburgh, PA) has had an increased number of overdoses and deaths due to fentanyl. Fentanyl, a synthetic opioid has been utilized for its analgesic/anesthetic properties since the early 1960s. Until recently its use for subjective (non-analgesic) purposes was primarily the abuse of pharmaceutical preparations. Due to fentanyl's enhanced potency (~80 x morphine) it has only been available for transdermal or buccal drug delivery for unsupervised patient use. In early 2006 (April/May) a rash of overdoses and several deaths occurred in the Pittsburgh (Allegheny County) area ultimately attributed to fentanyl not originating from a pharmaceutical product. The fentanyl was packaged and being sold "on the street" in the same manner as heroin. Within 3 days there were 5 deaths and more than 40 overdoses. One thousand packets of drug were seized by investigators and submitted to the Laboratory for analysis. In 2006 there were 27 deaths attributed to fentanyl alone, or in combination with heroin.

Post-mortem blood fentanyl concentrations ranged from 3.4 ng/mL to 113 ng/mL, with a heart blood to femoral blood ratio ranging from 1.1 to 2.3 (n=15). Concentrations of other drugs present will be presented. The most commonly found drugs were alprazolam, cocaine metabolite, and morphine. Packets of drug submitted to the Laboratory and analyzed contained 20 to 70 mg of white powder. Of the more than 100 drug packets ("bags" or stamp bags) submitted, approximately 50% contained heroin combined with fentanyl, and 50% contained only fentanyl. Only several packets contained cocaine with either fentanyl, or fentanyl and heroin. The packets containing fentanyl were either unmarked or were stamped with various phrases or words e.g. "Get High or Die Trying," "Burnout'" "Way to Go/Dondiva," "Tiffany," "Fendi," and "Truth" as examples.

Analytical methods used for identification and quantitation included immunoassay (Elisa) testing and gas chromatography/mass spectrometry (GC/MS). The GC/MS method involved liquid/liquid extraction, deuterated (fentanyl-d5) internal standard and SIM ions 146, 189, and 245 for fentanyl. Information and data from death cases and forensic drug identification cases will be presented.

Keywords: **Fentanyl, Illicit, Pharmaceutical**

P20 Homicide and Drugs in Puerto Rico 2006

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We will present statistics about the distribution of drugs of abuse in homicide cases during the year 2006 to determine the incidence of drug present in homicide cases in Puerto Rico. The drugs included in this study were cocaine/crack, opiates and cannabinoids in blood and urine. The information obtained will be classified by type of drug, age group and gender.

The data was generated by performing preliminary tests in the samples using ELISA (Enzyme-Linked ImmunoSorbent Assay) for screening and confirmation by GC/MS after extraction using automated SPE (Solid Phase Extraction).

We evaluated results obtained from 713 cases, of which 50% were positive for one or more of the drugs considered.

Results among the age group 30 to 39 years showed that 50% of the cases were positive for cocaine/crack. The same group age has a greater incidence in opiates (26%). On the other hand, the greater incidence for positive of THC was found among the ages 0 to 19 years (51%).

The results confirmed that a high percent of homicides in our jurisdiction are related to drug use.

Results obtained when divided by gender:

Gender	Analyzed Cases	Cocaine Positives	Opiates Positives
Male	674	231	106
Female	39	6	2

Gender	Analyzed Cases	THC Positives
Male	476	171
Female	22	3

Results obtained when divided by age groups:

Age group	Analyzed Cases	Cocaine Positives	Opiates Positives
0-19	82	16	4
20-29	366	107	49
30-39	142	71	37
40-49	65	30	12
50-59	24	6	2
60+	27	1	0

Age group	Analyzed Cases	THC Positives
0-19	43	22
20-29	267	115
30-39	103	28
40-49	46	6
50-59	16	0
60+	17	0

Keywords: **Homicide, Drugs, Statistics**

P21 Establishment of a Drug Standard Reference Library for Postmortem Toxicology Using Direct Analysis in Real Time (DART™) Time-of-Flight Mass Spectrometry (TOF-MS)

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The TOF-DART™ was utilized to identify over 100 compounds. The DART™ ion source allowed for the analysis of these compounds with little or no sample preparation and the TOF-MS allowed their identification by exact mass and fragmentation.

As part of a larger project to evaluate the application of the TOF-DART to postmortem toxicology screening (NIJ Grant 2006-DN-BX-K014), 111 drug standard compounds (drugs and metabolites) were assayed in solution to evaluate their ionization and detection of each using this system. Drug classes investigated included benzodiazepines, barbiturates, opioids, cocaine, amphetamines, antidepressants, cannabinoids, and hallucinogens. The data were stored in a library for identification and comparison purposes. The analyses were conducted using both positive (predominantly M+H ions) and negative modes (predominantly M–H ions) of the DART™ ion source. After both positive and negative analysis of the 111 standards compounds only 4 were unable to produce an M+H or M-H ion. These four were buprenorphine-glucuronide, 9-carboxy-11 nor-tetrahydrocannabinol, 9-carboxy-11 nor-D9 THC-glucuronide and psilocybin. All other compounds tested produced an expected M+H ion. Forty-nine compounds tested did not produce an expected M-H ion.

During the analysis, 15 potential isomer or interference pairs were identified. These pairs of compounds were exact isomers or had exact monoisotopic masses similar enough to be difficult to resolve due to isotopic contributions. These compounds were analyzed using different parameters to fragment each compound for identification and examine if characteristic fragmentation could be obtained. The analysis of isomer pairs such as cocaine and scopolamine was conducted in positive analysis mode by raising the voltage on the entrance orifice cone to produce fragments of each compound. Most isomer pairs were distinguishable by unique ions from fragmentation.

The TOF-DART™ successfully identified over 107 compounds commonly analyzed in postmortem testing from manufactured standard solutions. The data obtained from each analysis, including isomer fragmentation, are stored within the instrument's library search program for future comparison. The creation of these libraries enhances the TOF-DART™ by providing fast and reliable data analysis. Libraries generated in this manner could potentially be shared with other sites to promote standardization of future analyses between forensic laboratories.

Keywords: **DART™, TOF-MS, Exact Mass, Postmortem**

P22 Screening of Postmortem Specimens by Ultra Performance Liquid Chromatography and Time of Flight Mass Spectrometry

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Objective: The objective was to develop a novel qualitative method for the extraction and analysis of drugs of abuse and therapeutic drugs in postmortem whole blood, serum, plasma, gastric, tissue, and vitreous specimens by precipitation with Acetonitrile. The previous methods (ELISA and GC/MS) of screening postmortem specimens presented limitations of specimen run time, sensitivity, limited specimen volumes, and cost.

Method: A 250 microliter aliquot of specimen was precipitated with 1 milliliter of Acetonitrile containing an internal standard (Proadifen, 10 ng/mL). Specimens were vortexed, centrifuged, and supernatant was transferred into a 96-well plate. Ten microliters were injected for UPLC separation, which was performed by a Waters LCT Premier XE Time of Flight mass spectrometer coupled with a Waters Acquity UPLC system. The column was a Waters Acquity UPLC HSS T3, 2.1 x 100 mm, 1.8 µm particle size, held at 40˚C. Flow rate was 0.3 milliliters per minute. Mobile phases consisted of 0.05% Formic Acid in DI water and Optima grade Methanol. Initial mobile phase composition was 90% aqueous and 10% organic. Following a short hold, the gradient increased linearly to 95% organic and 5% aqueous. At 5 minutes, the gradient was brought back to initial conditions, for a total run time of 8 minutes. All specimens were held at 7.5˚C.

The mass spectrometer was operated in W-mode for higher resolution (> 10,000 FWHM). In order to detect both acidic and basic analytes, scans were completed in both positive and negative electrospray ionization modes. A low voltage scan (Cone Voltage = 30, Aperture Voltage = 10) was used for parent mass identification and a high voltage scan (Cone Voltage = 50, Aperture Voltage = 60) was used to identify parent mass fragmentation. To extend the linear range, dynamic range enhancement (DRE) was used. Real time accurate mass data was acquired by reference to an independently sampled reference material (Leucine Enkephalin, [M+H] = 556.2771 amu).

Results: A method was developed to screen for drugs of abuse and therapeutic drugs in postmortem specimens by precipitation with Acetonitrile. Specimen run time was reduced from 26 minutes for GC/MS to 8 minutes for LC/MS. The expanded library (220 analytes), as compared to GC/MS (120 analytes), allowed us to eliminate 10 of 13 ELISA assays. A parallel specimen study was performed between the ELISA, GC/MS, and LC/MS, with the LC/MS showing far superior results. No matrix effects were noted during the parallel specimen study. Detection limits varied for all major analytes.

Conclusion: A new UPLC/Time of Flight mass spectrometry method for the screening of postmortem specimens was developed. Specimen preparation was simplified and run times were significantly reduced when compared to the GC/MS method. The method has an expanded library of analytes, remarkable sensitivity and specificity, and significant cost savings. This method has proven to be far superior to the combination of ELISA and GC/MS methods of analysis.

Keywords: **Screening, UPLC, Time of Flight Mass Spectrometry**

P23 LC/MS Chromalynx: A More Comprehensive and Sensitive Screening For Post-Mortem Cases

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The wide array of drugs used in our society requires post mortem forensic toxicology laboratories to have a comprehensive and sensitive screening method. There are a large number of cases where several drugs are being used. The results provided by the laboratory are of utmost importance for the pathologist's determination of cause, manner, and circumstances of death.

The purpose of this paper is to present a comprehensive and sensitive screening method using LC/MS and the Chromalynx library. Waters Alliance 2695 HPLC with a ZQ Mass Spectrometer with a 2.1mm x 150mm and 3.5µm XTerra C_{18} column at 30ºC was used for the analysis. Analyses were performed using a gradient in wich solution A was a solution of ammonium formate / formic acid at a pH of 3 and solution B was acetonitrile/ with 0.1% formic acid. The gradient run was from 95A and 5B for the first 2 minutes to 10A and 90B at 16minutes returning to the initial gradient for a total run time of 26 minutes. An optimized automated SPE using UCT Clean-Screen CSDAU203 columns with Zymark Rapid Trace modules is proposed so that both acid and basic drugs may be detected on a single run.

The Institute of Forensic Sciences toxicology laboratory has been using the REMEDI system for several years. This system proved to be a tool for screening but lacked the sensitivity needed for the analysis of some drugs, especially in blood.

This paper will present the most common drugs encountered in our cases from July 2005 to May 2007, many of which were sent to a reference laboratory for confirmation and quantitation. We will present data obtained by a reference laboratory, ToxiLab analysis and compare it to data obtained with LC/MS and the Chromalynx library.

Chromalynx Library includes 500 drugs and metabolites with the capability to add more analytes by the user. We analyzed approximately 70 drugs; the analysis process of various examples of the most commonly encountered drugs in our population will be shown. Among the drugs analyzed were drugs of abuse and prescription drugs such as: benzodiazepines, barbiturates, zolpidem, fluoxetine, bupropion, carbamazepine and paroxetine. Their confirmation by comparison with certified primary standards will also be presented.

Keywords: **Chromalynx, Screening, Sensitivity, Postmortem**

P24 LC/MS (TOF) Analysis Of Benzodiazepines In Urine From Alleged Victims Of Drug Facilitated Sexual Assault

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The present study employs a recently reported liquid chromatography/mass spectrometry (time of flight) LC/MS (TOF) procedure for the simultaneous analysis of 22 benzodiazepines in human urine specimens. The analysis focused on the most commonly prescribed benzodiazepines and/or their metabolites which were extracted from urine after enzymatic hydrolysis using chloroform:isopropanol (9:1) followed by evaporation and reconstitution in methanol. Using this method, the LOQ for the benzodiazepines tested ranged from 2 to 10 ng/mL, while the LOD range was 0.5 to 3.0 ng/mL. Urine specimens collected from alleged victims of drug-induced sexual assault (156 specimens) were tested. Only 19 out of the 22 benzodiazepines analyzed were detected in these specimens. These same specimens were previously screened for benzodiazepines by various immunoassay techniques using a 50 ng/mL cut off level and confirmed by a GC/MS method after acid hydrolysis to their benzophenone skeletons thus making the identification of the specific benzodiazepine (s) involved impossible for most specimens.
This study aims to offer an alternative methodology which would allow such identification for similar specimens. Additionally, the distribution of the individual benzodiazepines of interest among the 156 specimens as well as their prevalence in specimens originating in different U.S. states is presented.

Keywords: **Benzodiazepines, DFSA, Urine**

P25 An Overview of Drug Facilitated Sexual Assaults in New Jersey

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The NJ State Police – Office of Forensic Sciences (OFS) recognized DFSA cases as being in a class of their own in the mid 1990's. In 1999, the Central Regional Laboratory implemented the more specialized and in depth analysis of this type of case, which included GHB/GBL analysis. A compilation of data for the years 2003-2006 will be reviewed.

The DFSA Unit uses EMIT, ELISA, and GC/MS as screening techniques. GC/MS and LC/MS are implemented for confirmation purposes. Headspace GC/FID is used for blood alcohol, volatile and GHB/GBL analysis.

The number of cases submitted to the OFS has increased yearly. The number of positive cases remains at approximately 75%. In 2006, 140 DFSA cases were analyzed, 106 were positive. Ethanol only cases were the most prevalent (31%), ethanol and drugs (25%), polydrug (25%), and single drug (19%). Marijuana (24%) and cocaine (22%) were consistently in the top three drugs found in DFSA cases. Other drug classes commonly encountered in 2006 DFSA cases were: benzodiazepines (19%), narcotic analgesics (24%), anti-depressants (10%) and over-the-counter preparations (18%). Although flunitrazepam has not been seen in NJ since 2003, alprazolam (12%) was the most frequently encountered benzodiazepine. In the narcotic analgesic class, heroin metabolites (7%), and methadone (5%) were the most common, surpassing oxycodone from previous years. The most common anti-depressant last year was citalopram. The anti-histamine, diphenhydramine (11%) has obtained top five status overall since 2005.

Ninety-seven percent of DFSA submissions reported female victims. Of those cases 66% were between the ages of 15-24 and 67% were Caucasian. Camden County (13%) had the highest submissions within NJ followed by Atlantic (11%) and Cape May (9%). These numbers were consistent with earlier years. Camden is in close proximity to Philadelphia and is known for its high crime. Atlantic and Cape May counties consist of highly frequented shore communities, which include Atlantic City and Wildwood. Data has shown an increase in DFSA submissions in August and September with the winter months being lowest.

Keywords: **DFSA, Statistics, New Jersey**

P26 Two Incidences of Xylazine Use in New Jersey

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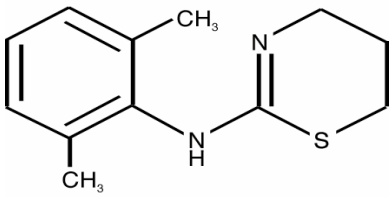
Two cases involving xylazine (Xyla-Ject®), a veterinary sedative/anesthetic/analgesic are presented. In the first case, xylazine was detected in a drug facilitated sexual assault (DFSA) case, as well as benzoylecgonine and THC/THCC. The second case was a suspected driving under the influence of drugs (DUID) case, and only xylazine was detected. Drowsiness, muscle flaccidity, loss of motor skills, and irrational behavior, were some of the observations by the arresting police officers and medical staff. Xylazine was screened and confirmed by GC/MS. The amount of xylazine detected in both cases is unknown since the NJSP does not perform quantitative analyses of drugs.

Case #1 – Late August 2005, approximately 24 hours later, the victim reported the DFSA to the police. She indicated that the previous evening she had consumed one and a half beers with the suspect at her residence then fell asleep. The following morning, unable to walk, the victim fell. She remained in that state until her daughter found her and transported her to the hospital. She felt that sexual contact had occurred with no memory of the incident. She believed that the suspect had put something in her drink. Initially, a urine sample was taken and an hour later, a blood sample. Toxicological analysis revealed xylazine, THCC, and benzoylecgonine in the urine, and THC in the blood. There was an insufficient amount of blood for ELISA and GHB/GBL analysis. Complete analysis of a DFSA specimen is as follows; blood alcohol concentration, volatile screen, GHB/GBL, EMIT and ELISA screens, GC/MS and LC/MS.

Case #2 – Mid September 2005, early in the evening police responded to a motor vehicle accident. The suspect ran into a wooded area off a state road and was apprehended by an officer for questioning. The suspect was given a Breathalyzer resulting in a reading of 0.00. Based on observations of the suspect at the scene and at the station, the officers believed the suspect was under the influence of drugs. SFST balance tests were not performed due to the fact that the suspect could not stand by himself and was unable to keep his eyes open. The suspect signed a consent form for blood analysis and was transported to a local hospital. Toxicological analysis revealed the presence of only xylazine in the blood. The blood sample was analyzed for volatiles, GHB/GBL, and drugs.

The confirmation for xylazine was performed using a SPE on an Agilent 6890N/5973 inert GC/MSD with a HP-5 30m column. The instrument parameters were; 250’C injection port, 280’C detector, Split 20:1. The temperature program used was 110’C-220’C@40’C/min-300’C@10’C/min (4min). The major ions of interest are: 205(100), 220, 177, 145, 130, 77.



Xylazine Structure

Keywords: **Xylazine, DFSA, Driving Impairment**

P27 Drug Impaired Driving in New Mexico-A Retrospective Study for the Years 2000-2006

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Driving under the influence of drugs accounts for a significant number of DWI cases in New Mexico. Each year the Scientific Laboratory Division receives between 4000-5000 cases from living and deceased persons. These cases involved traffic fatalities submitted from the Office of the Medical Investigator as well as persons arrested for DWI which were submitted by law enforcement. Retrospective data collected from the years 2000 to 2006 was correlated to identify the overall trends. Currently, our methodology measures BACs, ELISA screening, and presumptive positive confirmations by GC/MS.

Of the total number of cases reported during the years 2000-2006, 61% were fatally injured persons involved in traffic crashes. These cases had either drugs or alcohol or both. The most frequently detected drugs included stimulants and cannabinoids. In the Office of Medical Investigator cases we had a total drug mention of 1582 cases. Of these cases 78.5% (n=1242) contained ethanol of 0.08g/100mls or more and 22% contained drugs other than alcohol. The number of cocaine and methamphetamine drug mentions increased to 170% and 98% respectively over the years searched. Our total drug mentions from this data produced exceeded numbers to our total number of cases due to multiple drug use. This number was 54%.

In our DUID cases reported the most common drugs included cannabinoids, cocaine and methamphetamine. The age of persons stopped was between 22-30 years old and approximately 50% involved a traffic crash as the primary reason for the initial stop. Most of the stopped drivers were erratic driving (weaving, speeding, failure to yield) and this accounted for 41%. Great bodily injury was 6%, and only 3% had a fatality involved.

Driving under the influence of drugs or alcohol accounts for a significant number of the DWI cases in New Mexico. The number of drugs seen is indeed rising as an indication that the problem is growing rapidly by the year.

Keywords: **Driving Impairment, Alcohol, Drugs**

P28 Concentrations of Amphetamine, Methylenedioxymethylamphetamine (MDMA), Cocaine and Benzoylecgonine (BZE) in Whole Blood Samples Collected From Drivers in the United Kingdom, Suspected of Driving Whilst Under the Influence of Drugs

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This paper provides a summary of the concentration ranges of amphetamine, MDMA, cocaine and BZE, detected in whole blood collected from drivers. The data is presented as plots of percentage of cases versus blood concentration, together with the percentage of cases encountered at individual drug concentrations.

The paper outlines the toxicology results from 1585 cases of alleged driving whilst impaired and/or under the influence of drugs analysed at the Forensic Science Service (FSS) laboratory, Chorley between March 2001 and April 2003. Amphetamine was detected in 17% of cases, MDMA was detected in 13% of cases and cocaine and/or BZE was detected in 27% of cases.

Confirmatory and quantitative analyses using Gas Chromatography Mass Spectrometry (GC/MS) was undertaken, following preliminary screening, using Enzmye Immunoassay (EIA) and automated Solid Phase Extraction (SPE).

Data derived from cases involving amphetamine (n=256), MDMA (229), methadone (110) and BZE (121) are presented. The analysis of data in this format can assist forensic toxicologists to provide interpretative evidence in court, in a manner that can be easily understood by a jury.

Keywords: **Driving Impairment, Amphetamine, MDMA, Cocaine**

P29 A Method for the Estimation of Drug Concentrations in Dried Bloodstains

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Dried blood is a commonly encountered specimen type in both the clinical and forensic laboratory. In the clinical environment dried blood is an ideal specimen choice due to the ease of its collection, transport and storage. It is used for several types of tests including screening newborns for the detection of certain inherited genetic disorders such as Hepatorenal tyrosinemia and Niemann-Pick, evaluating lipid levels (e.g., Gaucher disease), in drug discovery testing and assessing lead exposure. In the forensic environment, dried blood is most commonly used to determine serological markers such as blood grouping (ABO) and sub-grouping, enzyme polymorphisms and for DNA testing purposes. Less frequently, dried blood can be used for the detection of drugs and their metabolites, metals and anticoagulants. Most of these analyses are either qualitative or the final quantitative result is based upon an assumed volume of blood. Here we report an alternative method for the estimation of drug quantity by normalizing the concentration of drug and drug metabolite(s) to the amount of iron (Fe) found in the dried blood. We present a case to illustrate the use and application of this method.

In our analytical protocol, two near equivalent sections of material, one containing dried blood and the other appearing to be negative for blood, underwent a one-step extraction process with methanol. The methanolic extracts were screened by Enzyme Multiplied Immunoassay Technique (EMIT) and any confirmation work performed by Gas Chromatography-Mass Spectrometry (GC/MS). The iron concentrations in the sample and control areas were determined by performing an acid digestion on the material followed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

Some of the assumptions that were made include the following: 1) The blood is from a single individual, 2) The drug and metabolites come only from the blood and not from local surface contamination or sweat, 3) The blood is uniformly distributed over the stained area, 4) Any background iron is uniformly distributed throughout the entire material, 5) The individual is of normal hematological status (i.e. neither anemic nor polycythemic), 6) The compounds of interest are stable in the dried blood matrix and will not appreciably change in identity (i.e. cocaine will not degrade to benzoylecgonine).

The amount of iron in the blood stained area, normalized to a gram of cloth, when corrected for background iron was 1177 µg iron/g of cloth. Given that in healthy adult males the amount of iron in whole blood is in the range of 440 to 625 mg/L, then a blood stained cloth weighing 697 mg would have a blood volume between 1.31 to 1.87 mL.

The results in this case were (with the estimated concentration range), cocaine 364 ng (195 to 277 ng/mL), cocaethylene 25 ng (13 to 19 ng/mL) and benzoylecgonine 623 ng (334 to 474 ng/mL).

Keywords: **Dried Bloodstains, Drug Analysis, Iron (Fe)**

P30 A Rapid Method for the Analysis of 12 Benzodiazepines in Blood Using GC/MS

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A gas chromatographic/mass spectrometric (GC/MS) method was developed for the simultaneous quantitation of 12 benzodiazepines in whole blood. This procedure incorporates a United Chemical Technologies Styre Screen™ polymeric solid phase extraction (SPE) followed by derivitization with BSTFA w/1%TMCS. All sample volumes extracted were 3 mL spiked with a corresponding deuterated analogue with the exception of midazolam where deuterated temazepam was used. The GC/MS was run in SIM mode with a dwell time of 30 ms. Five ions were monitored for each benzodiazepine, three were used for quantitation and confirmation and two were available as backup ions. The method described is highly selective and sensitive with limits of detection (LOD) ranging from 1.56-6.25ng/mL. Linear dynamic ranges (LDR) varied from 1.56-3200ng/mL using calibration curves weighted by a factor of 1/x. The SPE provided sufficient sample extraction yielding recoveries of 25-37% and 47-76% at 20 and 200ng/mL respectively (n=5 for each group). Furthermore, the developed procedure provided superb accuracy and precision. This procedure showed intra-day (within day) relative errors between 1-16% and relative standard deviations (RSD) less than 3% for both the 20 ng/mL and 200 ng/mL control groups (n=5 for each group). Using whole blood controls stored at 4ºC the inter-day (between day) relative errors for the 20 ng/mL control group were between 3 and 17% for days 2 and 6, respectively (n=5). Relative errors for the 200 ng/mL control group were between 2 and 13% for days 2 and 6, respectively (n=5). The RSDs were < 4% for both control groups over the 6-day period. Based on the day 6 results, it is clear that these 12 benzodiazepines are stable in blood stored at 4ºC. The method developed proved to be rapid, reliable and sensitive for the identification and quantitation of 12 benzodiazepines in whole blood.

Keywords: **Benzodiazepines, GC/MS, Polymeric Solid Phase Extraction**

P31 A Comprehensive Analysis for Benzodiazepines in Biological Matrices Utilizing LC-MS/MS Following a Class-Specific Solid Phase Extraction

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An extensive confirmation method for benzodiazepines in blood, serum and tissue has been developed using a class-specific solid phase extraction (SPE) followed by LC-MS/MS analysis. Blood, serum, and tissue samples were spiked with deuterated internal standards (specifically d5-diazepam and d5-alprazolam) at 0.50 mg/L and then extracted using UCT CLEAN SCREEN® Extraction Columns according to laboratory protocol. The SPE column is first prepped sequentially with methanol, deionized water, and 0.1M sodium phosphate buffer, pH 6.0. The sample is then added to the column and pulled through under vacuum. Prior to elution with ethyl acetate, the column is washed first with deionized water and then 20% acetonitrile in 0.1M phosphate buffer. Dried down extracts were reconstituted with 150 mL diH2O. The analytes of interest include diazepam, nordiazepam, temazepam, oxazepam, chlordiazepoxide, desalkylflurazepam, lorazepam, clonazepam, 7-aminoclonazepam, and 7-aminoflunitrazepam using d5-diazepam as internal standard, and alprazolam, alpha-hydroxyalprazolam, midazolam, estazolam, and triazolam using d5-alprazolam as internal standard.

Analyses were performed on a Varian ProStar® HPLC with a Model 410 Autosampler coupled to a Varian 1200L Quadrupole MS/MS. 40 mL of sample were injected onto a Waters Symmetry® C8 3.5 mm 2.1 x 150 mm column with a Waters Sentry® 2.1 x 10 mm guard column. The solvent gradient ramped from 70:30 0.1% formic acid:acetonitrile to 20:80 0.1% formic acid:acetonitrile at 0.200 mL/min followed by a 1.5 minute organic flush and 6-minute column re-equilibration, also at 0.200 mL/min. Analytes were detected using parent ions (m+1) and a selected, unique daughter ion at an optimized collision energy.

Quantitation of the analytes was based on parent/daughter ion ratios from mid-level whole-blood extracted calibrators in the range of 1.0 mg/L to 2.0 mg/L. This covers the sub-therapeutic range of these prescription drugs up to the slightly toxic levels of the drugs. Due to the fairly large concentration range and expected matrix interferences, a quadratic fit was used for calibration curves. The limit of detection for the analysis is 1.0 mg/L while the limit of quantitation is 5.0 mg/L. The standard deviation for intra-run variability of separately prepared controls is less than 0.008 for low controls (0.075 mg/L), less than 0.026 for mid controls (0.250 mg/L), and less than 0.095 for high controls (0.750 mg/L). At these same levels, the inter-run variability standard deviation is less than 0.010, 0.057, and 0.138, respectively. Ion suppression studies revealed that suppression of monitored ions was negligible, generally less than 11% at low-end controls (0.010 mg/L) and this occurrence decreases with increasing concentration. This class-specific extraction does allow for additional deuterated internal standards, which will be considered pending further studies.

Keywords: **Benzodiazepines, LC/MS/MS, Solid Phase Extraction**

P32 Quantitative Determination of Olanzapine in Whole Blood Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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Introduction: Olanzapine is an antipsychotic drug used to treat schizophrenia and related disorders. Olanzapine is usually administered in very low doses (5-20 mg/day) and the plasma levels are correspondingly low (0.003-0.1 mg/l). Since, olanzapine binds strongly to proteins an effective sample preparation is needed to isolate olanzapine from whole blood samples. The present work demonstrates a simple, reproducible and sensitive method for quantification of olanzapine in whole blood from forensic cases using simple protein precipitation and liquid chromatography-tandem mass spectrometry (LC-MS/MS). To prevent oxidation of olanzapine during sample preparation ascorbic acid is added to each sample as a stabilizing agent, and all samples and standard solutions are protected from sunlight during storage and sample preparation.

Methods: 0.200 g whole blood is mixed with 2 µl 25 % ascorbic acid. An internal standard (dibenzepine) is added and the sample is precipitated with methanol at low pH. After centrifugation at low temperature the supernatant is evaporated and the residue is dissolved in 200 µl solvent. Spiked whole blood samples at 5 levels between 0.005-0.50 mg/kg are used for the calibration curve. Chromatographic separation (HP 1100 series, Agilent Tech.) of olanzapine from endogenous blood interferences and other drugs is accomplished with a Zorbax Extend-C_{18} column (50 mm x 2.1 mm id., 5 µm). The solvents used are A: 5:95 acetonitrile:ammonium hydroxide (5 mM) and B: acetonitrile using gradient elution with a flow rate of 0.2 ml/min. The initial condition of 3% B was increased to 80% at 10 min and held for 0.5 min before returning to initial condition. Total time of analysis is 15 min. Detection is performed by positive electrospray ionisation with a triple quadrupole mass spectrometer (Quattro micro, Waters) operating in multiple reaction monitoring (MRM) mode. The MRM transitions are *m/z* 313 → 256 and *m/z* 313 → 84 as the quantitative and confirmative traces, respectively.

Results: All 5 calibrators and in house control samples at low and high level are measured in each series. Calibration curves show linearity in the range from 0.005 to 0.50 mg/kg with correlation coefficients (r^2) above 0.99. The limit of detection (LOD) and quantification (LOQ) are estimated at 0.001 and 0.005 mg/kg, respectively. Precision, expressed as coefficient of variation (CV), is estimated to less than 5 % at both low (0.01 mg/kg, n=6) and high level (0.50 mg/kg, n=8). The accuracy is within 103-115 % at low level and within 98-116 % at high level. The absolute recovery of olanzapine from whole blood obtained is approximately 99 %.

Conclusion: A simple, sensitive and reproducible LC-MS/MS method using protein precipitation is developed to extract olanzapine from whole blood. The method is used to analyze olanzapine in authentic blood samples from forensic cases.

Keywords: **Olanzapine, Blood, LC/MS/MS**

P33 Analysis of Antipsychotic and Antidepressants in Whole Blood by LC/MS/MS

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The objective of this research was to develop an LC/MS/MS method for screening and confirmation of various antidepressant and antipsychotic drugs in whole blood. These types of compounds are consistently among the most commonly prescribed medications and as such can be routinely encountered in the performance of therapeutic drug monitoring or medical examiner toxicological examinations. The analysis of these types of drugs may be hampered by laborious extraction procedures, extended analytical analysis times, and even the need for multiple analyses. A generalized sample preparation and analysis method by HPLC tandem MS is presented.

This method describes the simultaneous analysis of more than 10 antipsychotic, antidepressant and structurally similar medications. The drugs analyzed for include amitriptyline, imipramine, desipramine, clomipramine, olanzapine, fluvoxamine, paroxetine, sertraline, fluoxetine, mianserin, trazodone and haloperidol. Whole blood is subjected to protein precipitation. The corresponding supernatant was subsequently analyzed using reversed-phase HPLC with MS/MS detection. The column used was an Allure PFP Propyl from Restek Corporation. The mobile phase consisted of a binary mixture for a gradient. Mobile phase A was aqueous 1.0 mM ammonium acetate with 0.05% acetic acid. Mobile phase B was 95% Acetonitrile and 5% water with 1.0 mM ammonium acetate and 0.05% acetic acid. Detection was by single reaction monitoring for each compound.

All of the analyzed antipsychotics, antidepressants and structural analogs were analyzed in a single method. The analytical run time was complete within 15 minutes. Detection limits of the individual drugs and their observed linear ranges are presented. Linear ranges for the drugs generally covered at least two orders of magnitude. The limits of detections for many the compounds allow for application of the method to monitoring therapeutic concentrations.

The method provides an accurate and reliable means for detection and confirmation of various antipsychotic and antidepressant drugs in a whole blood matrix. The use of a precipitation as a means of sample preparation is less laborious and more time effective than classical liquid-liquid or solid phase extraction. In addition the versatility associated with HPLC and tandem mass spectrometry makes it likely that addition drugs could be added to expand the scope of the analysis with few modifications.

Keywords: **Blood, Antidepressants, LC/MS/MS**

P34 Emergences of Xylazine as an Adulterant of Heroin in Puerto Rico

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Xylazine is a veterinary sedative, analgesic or general anesthetic that has been proven harmful to humans. In 1960, xylazine was investigated as a sedative-hypnotic/analgesic premedication in humans, but was rejected because of its frequent association with severe hypotension. Eighteen cases of toxicity caused by xylazine consumption via oral or parenteral administration (intramuscular, subcutaneous, and intravenous) have been documented in humans. In these cases, consumption was either involuntary, suicidal or homicidal purposes, or used as an agent of drug abuse, occasionally resulting in death. Nowadays, xylazine is being used as an adulterant according to the street heroin samples analyzed by the Control Substance Laboratory of the Institute of Forensic Sciences of Puerto Rico. In 2006 a total of 997 heroin cases were examined. Xylazine was found in 633 (36%) cases as an adulterant of heroin. Due to its toxicity in humans, and the fact that it is found in street drugs, an automated solid phase extraction method is being developed to detect xylazine in human blood using GC-MS SIM as the method of detection to establish the appearance of Xylazine in heroin overdose cases.

A basic drug extraction method was selected for the analysis of xylazine. Prazepam was added as an internal standard to a 2 mL sample. The sample was diluted with 0.1 M phosphate buffer (pH 6.0), vortexed, and pH adjusted to 6.0 ± 0.5 with 0.1 M monobasic or dibasic sodium phosphate. The analyte was diluted and collected with 3 mL elution solvent [CH_2CL_2/IPA/NH_4OH (78/20/2)]. The eluted sample was evaporated to dryness at ≤ 70°C under a gentle stream of nitrogen, reconstituted with 100 µL of methanol and 2µL was injected into the GC-MS.

The GC-MS system used was an Agilent 6890 Series GC System coupled with Agilent 5973 Network mass selective detector. Helium was used as a carrier gas at a flow rate of 1 mL/min through the DB-5MS capillary column 15-m long, 0.25-mm i.d. and 0.25-µm film thickness. The initial temperature was held at 120°C for 2 min and then increased to 270°C at a rate of 30°C per minute. The total run time was 9 min. The injector temperature and the transfer line were set at 220°C and 270°C, respectively.

Keywords: **Xylazine, SPE, GC/MS**

P35 Evaluation of the Lin-Zhi International Propoxyphene Enzyme Immunoassay for the Detection of Propoxyphene and Its Norpropoxyphene Metabolite in Urine

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We present an evaluation of a new Propoxyphene Enzyme Immunoassay [PEI] (Lin-Zhi International, Inc., Sunnyvale, CA) for the detection of propoxyphene (PX) and its major metabolite, norpropoxyphene (NPX), in urine. The Lin-Zhi assay is based on competitive antibody binding between PX in urine and glucose-6-phosphate dehydrogenase labeled PX. When PX and/or NPX are present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm. The assay is calibrated with 300 ng/mL of PX and a stated cross-reactivity of NPX is 620 ng/mL.

The PEI was evaluated by testing 1409 urine specimens collected from pain management patients presently, or in the past, prescribed PX. All 1409 specimens were tested with the assay in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY) with calibrators containing 0, 300 (cut-off calibrator) and 600 ng/mL of PX. Controls containing 0 ng/mL of PX and -25% (negative control) and +25% (positive control) of the 300 ng/mL cut-off calibrator (Bio-Rad Laboratories, Irvine, CA) were analyzed with each batch of samples. All specimens were also tested with the DRI PX Assay (Microgenics, Sunnyvale, CA) [DRI] according to the manufacturer's recommendations. All urines were then analyzed by gas chromatography /mass spectrometry (GC/MS) for PX/NPX at a cut-off concentration of 100 ng/mL.

Approximately, 64% (899) of the 1409 specimens yielded positive results by the PEI assay. GC/MS confirmed the presence of PX and/or NPX at $\geq$100 ng/mL in 98% of these specimens, indicating twenty false positive results. However, 98 specimens yielding negative PEI results were found to contain NPX above the GC/MS cut-off of 100 ng/mL. Therefore, the overall agreement of PEI and GC/MS results was 92%. Determined by GC/MS, PX and NPX concentrations in the specimens ranged from 154 -13,500 ng/mL and 200 – 60,000 ng/mL, respectively. From the presented study, the sensitivity of the PEI was 0.900 and the selectivity 0.954. The DRI assay yielded a sensitivity of 0.887 and selectivity of 0.960. The low selectivity for NPX by both immunoassays resulted in the high number of false negatives. The PEI assay demonstrated no cross reactivity at 1,000 ng/mL with common drugs of abuse. The within-run and between-run precisions of the PEI assay as determined by the absorbance rates of the negative and positive controls were CV=1% (n=18) and CV=<5% (n=18), respectively. The assay was found linear from –50% to 150% of the cut-off concentration. The Lin-Zhi PEI yielded results comparable to those of other popular enzyme immunoassays and provides a reliable method for the routine detection of PX and/or NPX in urine specimens.

Keywords: **Enzyme Immunoassay, Propoxyphene, Urine Drug Testing**

P36 Methadone and its Major Metabolite, EDDP, Identification and Quantitation Using GC/MS-Positive Chemical Ionization

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Methadone is an Opiate Agonist utilized for Heroin addiction intervention but recently has been prescribed in generalized pain management. Assays for this drug agent in the Toxicology Laboratory are based on the presence of the parent drug, which has been proven problematic with respect to the SIM assays, since the base peak in the Electron Impact mass spectra represents an alkyl amine moiety (m/z 72). In order to address those problems it is therefore necessary to choose ions of low intensity for monitoring purposes, or assays have been established based on the metabolite EDDP. The development of this method we believe has circumvented these problems by allowing us excellent signal to noise ratio of m/z 310 (M+1) at very low concentrations.

Five milliliters of blank urine or one milliliter of blood are the specimens requirements,. A pH of 9.3 was optimal for Liquid/Liquid extraction, and was attained with 1.0 M Sodium Phosphate Buffer. The optimal extraction was attained with a solvent comprised of 50% Toluene, 30% ethyl acetate, 15% Butyl Chloride (Blood), 15% Hexane (Urine) 5% Isopropanol. The LOD of 0.5ng/ml and LOQ of 1 ng/mL for positive chemical ionization with flow of 20 psi methane gas, were achieved repeatedly for parent Methadone and EDDP metabolite. Upper limits of linearity are routinely greater than 4,000 ng/mL. In the developmental phase of this method all runs were repeated in triplicate and a precision of 0.5% was attained. The optimized GC/MS-PCI method run on Agilent 6890/5973N-PCI was found to elicit results that were far superior to the Electron Impact Fragmentation method in sensitivity, specificity (Methadone molecular ion monitoring), and laboratory overall ruggedness. We have demonstrated absolutely no generation of EDDP or EMDP from Methadone in this assay. This method affords us the quantitation of the molecular ion +1, m/z 310 for Methadone and m/z 278 for EDDP with comparable if not better performance than other methods found in published literature.

Keywords: **Methadone, EDDP, GC/MS-PCI**

P37 Validation of a LC-APCI-MS/MS Method for Quantification of Methadone, 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-Ethyl-5-methyl-3,3-diphenylpyraline (EMDP) in Infant Plasma Following Protein Precipitation

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Objective: A quantitative LC-APCI-MS/MS method for the rapid and simple determination of methadone and metabolites in plasma was developed and validated.

Method: Specimen (200 µL) preparation required only protein precipitation with acetonitrile (600 µL) and centrifugation prior to injection into the LC-MS/MS. An LCQ Deca XP Ion Trap Mass Spectrometer, equipped with an APCI source, was interfaced to a Surveyor HPLC system. A Phenomenex Synergi Hydro-RP analytical column provided optimum chromatographic separation with a gradient mobile phase of (A) 10 mM ammonium acetate in water with 0.001% formic acid and (B) acetonitrile with a gradient program of 40% B for 2 min, increasing to 90% over 7 min and hold for 2 min. The HPLC column was re-equilibrated for 6 min. The flow rate was 200 µL/min. Mass spectrometry data were collected in positive ion mode and the identification and quantification of analytes were based on selected reaction monitoring. The following transitions were monitored: *m/z* 310.9 to 265.3 for methadone, *m/z* 319.9 to 268.3 for methadone-D9, *m/z* 278.0 to 249.2 for EDDP, *m/z* 281.0 to 249.2 for EDDP-D3 and *m/z* 264.3 to 235.2 for EMDP.

Validation: The following criteria were used to evaluate the method: specificity, sensitivity, limits of detection and quantification, linearity, precision, accuracy, recovery, carryover effect, matrix effect, and stability. Method validation was accomplished in four days with four unique assays.

Results: Total chromatographic run time was 17 min. Stability of the LC method was evaluated by calculating retention time variabilities. The percent relative variation for retention times was ≤ 0.78 % for all analytes over 35 consecutive runs. Calibration by linear regression analysis utilized deuterated internal standards and a weighting factor 1/x. Linearity was achieved from 1-500 ng/mL for all analytes with limits of detection and quantification of 0.5 and 1 ng/mL, respectively. There was no endogenous signal for any analyte in six blank human plasma specimens, demonstrating good specificity for the method. In addition, no commonly used over-the-counter or abused drugs at a concentration of 1000 ng/mL interfered with the quantification of 4 ng/mL methadone, EDDP or EMDP. Intra- and inter-assay accuracy were ≥ 87.5% and intra- and inter-assay precision < 13.4% RSD for all analytes: parameters were tested at 4, 40 and 400 ng/mL. Recovery from plasma at 40 ng/mL was ≥ 87.5% for all analytes.

Conclusion: The assay provides simultaneous quantitative analysis of methadone and metabolites EDDP and EMDP in human plasma with an LOQ of 1 ng/mL with minimal specimen preparation.

Keywords: **Methadone, Plasma, LC-APCI-MS/MS**

P38 Determination of Oxycodone and Metabolites by High Performance Liquid Chromatography-Electrospray Ionization -Tandem Mass Spectrometry

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Oxycodone is an opioid agonist used in the treatment of moderate to severe pain. A highly sensitive method was developed to measure oxycodone and its metabolites, noroxycodone and oxymorphone, in human plasma. Deuterated oxycodone, noroxycodone and oxymorphone were used as internal standards. Liquid / liquid extraction was applied for sample preparation using n-butyl chloride: methanol (4:1) as organic solvent. High performance liquid chromatography interfaced by electrospray ionization to a tandem mass spectrometric detector (HPLC-ESI-MS/MS) was used for quantitation. A YMC ODS-AQ S 5 µm 2.0 x 100 mm column (Waters Corporation, Milford, MA) was used for separation. The mass spectrometer was a Thermo Scientific model TSQ Quantum. Quadrupole 1 was set to pass only ions at m/z 316, 322, 302, 305, 302 and 305 that correspond to the MH^+ ions of oxycodone (316), oxycodone-d_6 (322), noroxycodone (302), noroxycodone-d_3 (305), oxymorphone (302) and oxymorphone-d_3 (305). The MH^+ ions were caused to undergo collision induced dissociation in quadrupole 2 that produced product ions at m/z 298, 304, 284, 287, 284 and 287 respectively, which were then monitored selectively by quadrupole 3. Noroxycodone and oxymorphone both have the same parent mass and daughter mass. Their identification was achieved by HPLC column separation. The calibration range was from 0.2 to 250 ng/mL with the calibration curve constructed as quadratic with 1/X weighting. Specificity for oxycodone, noroxycodone and oxymorphone was determined from analysis of blank plasma fortified with internal standard only (3 replicates) and with lower limit of quantitation (LLOQ) concentration (0.2 ng/mL) (1 replicate) in six different lots of plasma. The primary evaluation was to compare mean peak area ratio of any signal at retention time of the analytes to its internal standard for each lot with the mean peak area ratio of the six LLOQ samples. Mean ratios relative to mean LLOQ ranged from 0.92 to 16.6 with a mean of less than 11.4% for each analyte. Intra-run accuracy of the LLOQ was within 12.5% of target with intra-run precision within 6.2%. Intra- and inter-run precision and accuracy were also evaluated at 0.6, 10 and 200 ng/mL. The intra-run accuracy was within 10.8% of target with intra-run precision within 12.8%. The inter-run accuracy was within 5.0% of target with inter-run precision within 10.2%. All analytes were stable in human plasma for up to 24 hours at room temperature and after 3 freeze-thaw cycles. The mean extraction efficiency for oxycodone, noroxycodone and oxymorphone was 75.6%, 37.4% and 18.2% respectively. The results of these studies show that oxycodone and its metabolites can be quantified using liquid/liquid extraction and LC-ESI-MS/MS from 1-mL aliquots of human plasma. The method is very sensitive for pharmacokinetic studies and other clinical applications.

Keywords: **Oxycodone, HPLC-ESI-MS/MS, Plasma**

P39 Homogeneous Immunoassay for the Detection of Buprenorphine and Norbuprenorphine in Urine

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Background: Buprenorphine is increasingly being prescribed for the treatment of heroin addiction as an alternative to methadone. Due to its potency, the drug concentrations in body fluids are normally very low. Here, we report the first recombinant Glucose-6-phosphate dehydrogenase (G6PDH) based homogeneous immunoassay (EMIT-type assay) for free buprenorphine and free norbuprenorphine in urine.

Cross-reactivity: Because of its likely use with other opiates, potential interference in the assay is important. The antibody cross reacts almost 100% with free buprenorphine and free norbuprenorphine, and shows less than 1% cross reactivity with a wide range of opiates, particularly those structurally related compounds such as morphine, codeine and dihydrocodeine. Since other opiates may be present at high concentration, the specificity of the assay is essential.

Comparison of the specificity between new and currently available assays (CEDIA)

	New Assay		***CEDIA Assay***	
Buprenorphine (ng/mL)	**ΔmA/Min**	**Pos/Neg.**	**Pos/Neg.**	**ΔmA/Min**
0	388			388
2.5	435			428
5	470			457
10	554			512
20	621			640
40	635			921
1000	661			1492
Dihydrocodeine (100K)	388	N	P	488
Codeine (100K)	371	N	P	588
Naltrexone (100K)	383	N	N	419
Norpropoxyphene (100K)	420	N	P/N	455

Validation: The new assay has a low detection limit for free (non-conjugated) buprenorphine or norbuprenorphine (1 ng/mL). Further evaluation of this technique with GC/MS of authentic urine samples demonstrated that the accuracy was over 95%.

Summary: This highly specific assay is designed to measure the free drugs in urine, resulting in simplified confirmation methods, which don't require a hydrolysis step before extraction.

Keywords: **Buprenorphine; Norbuprenorphine; Opiate Cross-Reactivity**

P40 Fast and Sensitive Screening Methods for Buprenorphine, Norbuprenorphine and Their Glucuronides in Urine and Whole Blood by SPE and UPLC/TOFMS

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Objective: Buprenorphine (i.e. Subutex®, Suboxone® or Temgesic®) is an emerging drug in substitution therapy of opioid addicts in Denmark. Screening for buprenorphine by radioimmunoassay (RIA) has previously been applied in our laboratory, but the buprenorphine RIA kit is no longer available. Thus, the objective of this study was to compare four screening approaches for buprenorphine using UPLC/TOFMS.

Methods: *(1) Screening for buprenorphine and norbuprenorphine in urine after enzymatic hydrolysis and SPE* and *(2) Screening for buprenorphine and norbuprenorphine in blood after SPE*. Buprenorphine and norbuprenorphine were extracted by mixed-mode cat ion exchange SPE columns (Isolute HCX-3 (130 mg/3 ml)). Internal standards buprenorphine-d4 and norbuprenorphine-d3 were added to all samples *prior* to extraction. Urine (2 ml) was mixed with 1 ml 1 M acetate buffer (pH 5.5) and 25 µl β-glucuronidase/ arylsulphatase and hydrolyzed overnight at 40°C. Whole blood (0.5 ml) was mixed with 2.7 ml 0.1 M KH_2PO_4 buffer (pH 6), sonicated 10 min and centrifuged 10 min at 3600 rpm. Sample (3 ml) was loaded, followed by washing steps consisting of 1 ml 0.1 M KH_2PO_4 buffer, 1 ml 1 M acetic acid, and 1 ml methanol in consecutive order. After drying 10 min, the analytes were eluted with ammonium hydroxide (25% aq.) -acetonitrile -ethyl acetate (2:10:88, v/v). After evaporation at 45°C, the blood extract was reconstituted in 100 µl methanol and the urine extract was reconstituted in 2 ml 7.5% methanol.
(3) Screening for buprenorphine-glucuronide and norbuprenorphine-glucuronide in urine after filtration. Urine (0.25 ml) was diluted with 0.25 ml internal standard solution in methanol/water (1:1, v/v) and filtered through 0.2 µm Mini-UniPrep Filter (Watman, Clifton, NJ, USA).
(4) Screening for buprenorphine and norbuprenorphine in urine after enzymatic hydrolysis and filtration. Urine (0.25 ml) was diluted with 0.1 ml 1 M acetate buffer (pH 5.5), 25 µl β -glucuronidase/ arylsulphatase and hydrolyzed overnight at 40°C. The samples were diluted with 125 µl internal standard in methanol and filtered through 0.2 µm Mini-UniPrep Filter.

The UPLC/TOFMS conditions were UPLC (Acquity, Waters, Milford, MA, USA) 2.50 min gradient elution with water-formic acid (0.1%) and acetonitrile (80:20) to (60:40) on an Acquity BEH C18, 2.1 x100 mm column, 1.7 µm, flow rate 0.45 ml/min at 50 °C. The TOFMS (LCT Premier XE, Micromass, Manchester, UK) was operated in electropositive W-mode (ESI+).

Results: The mass accuracy was found to be better than 20 mDa. The linear range of the instrument methods were 0.0005 to 0.1 mg/l for buprenorphin-glucuronide, norbuprenorphine-glucuronide, and norbuprenorphine, 0.00005 to 0.1 mg/l for buprenorphine. Accuracy was verified by including spiked samples in each run.

LOD	Approach 1	Approach 2	Approach 3	Approach 4
Buprenorphine (-glucuronide)	0.0005 mg/l	0.0001 mg/l	0.001 mg/l	0.0005 mg/l
Norbuprenorphine (-glucuronide)	0.005 mg/l	0.001 mg/l	0,005 mg/l	0.005 mg/l

Urine from authentic living subjects and autopsy cases were applied for method comparison; 18 positive and 5 negative RIA-buprenorphine urine samples were confirmed by all screenings approaches on UPLC/TOFMS.

Keywords: **Buprenorphine, Screening, UPLC, Time of Flight Mass Spectrometry**

P41 LC/MS/MS Analysis of Buprenorphine, Norbuprenorphine and Their Glucuronides in Urine

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Background: Buprenorphine is increasingly being prescribed for the treatment of heroin addiction as an alternative to methadone. Rapid, sensitive confirmatory procedures are needed for the quantitation of the parent drug and conjugated metabolites in urine.

Methods: We present a rapid method for the detection of buprenorphine, norbuprenorphine and their glucuronides in urine involving simple dilution of authentic urine samples with deionized water. Two transitions per compound were monitored for the free drugs; one transition for the glucuronides. The monitoring of the qualifying ion and calculation of its ratio to the intensity of the primary transition provides additional information for forensic identification. To urine (0.1 mL), deionized water (0.35 mL) and 100 ng of internal standard were added.

LC/MS/MS Conditions: An Agilent LC 1200 Series pump connected to a 6410 triple quadrupole mass spectrometer operating in positive electrospray mode was used for the analysis. The column was a Zorbax Eclipse XDB C18 4.6 x 50mm x 1.8 μm, held at 40°C and the injection volume was 5 μL. The solvent flowrate was 0.8 mL/min.
The mobile phase consisted of 20mM ammonium formate (pH 6.4) as solvent A and methanol as solvent B. The phase was held at 40%A: 60% B for 2.5 min, then raised to 100% at 5 min where it was held for 3.5 min. The post time was 3 minutes. The gas temperature was 300°C; gas flow (N2) was 6 L/min; the nebulizer pressure was 50 psi and the capillary voltage 4500 V. The fragment voltage for all transitions was 240V.

Compound	*Precursor ion*	*Fragment ion*	*RT (min)*	*CE (V)*
D3-NBUP	417.4	399.3	1.16	40
NBUP 3 gluc	590.5	414.4	0.73	40
NBUP	414.4	340.4 (187.2)	1.17	35 (40)
D4-BUP	472.5	400.4	6.62	45
BUP 3 gluc	644.5	468.4	5.21	40
BUP	468.4	414.4 (396.1)	6.68	35 (55)

Summary: The method is simple, sensitive and rapid, with all analytes being determined in less than 8 minutes. The assay was precise and accurate and was linear over the range 5-100 ng/mL. The precision was less than 20% both within day and between days. The limit of quantitation was 5 ng/mL; the limit of detection was 1 ng/mL. The assay is suitable for the confirmation of buprenorphine, norbuprenorphine and their respective glucuronides in urine.

Keywords: **Tandem Mass Spectrometry, Buprenorphine, Conjugated Metabolites**

P42 An Expanded Opiate Panel for Urine Utilizing LC/MS/MS

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Objective: Analysis of hydromorphone, morphine, oxymorphone, 6-MAM, oxycodone, codeine, and hydrocodone in urine utilizing LC/MS/MS has previously been presented.[1] This method was extended to include the synthetic opioid fentanyl. Fentanyl is often analyzed in a separate assay from the other opiates because the required detection limit for fentanyl is about an order of magnitude lower than the required detection limit for the other opiates. This disparity presents not only a dynamic range mismatch, but a potential challenge for a technique to meet the low levels necessary for detection and quantification. The objective of this project was to develop a single assay that would detect and quantify all opiates, including fentanyl, in urine.

Method: An Agilent 1100 LC stack was interfaced to a hybrid triple quadrupole/linear ion trap mass spectrometer. Separation was achieved on a 2 mm x 50 mm Aquasil C18 column and total run time was 6.4 minutes. Mobile phases A and B were water and ACN, respectively, with 0.1% formic acid added to each.

Two MRM transitions, a quantifier and qualifier, were used for each analyte: hydromorphone, morphine, oxymorphone, 6-MAM, oxycodone, codeine, hydrocodone and fentanyl. Deuterated analogs of each were used as internal standards and spiked at 500ng/mL. One MRM transition for each internal standard was monitored. Sample preparation consisted of a simple hydrolysis step and dilution. A 250 µL aliquot of urine was hydrolyzed and diluted to a final volume of 2 mL. Ten microliters were injected for analysis.

Results: All eight opiates and their respective internal standards were successfully analyzed in a single LC/MS/MS method. The LOQ was about 0.25 ng/mL for fentanyl and 1 ng/mL for all other opiates. The linear dynamic range was from 5 ng/mL to at least 10000 ng/mL for all anlyates. Inter- and intra-assay precision were both measured. For inter-assay precision, five replicates each of the low and high QC standards were measured. For intra-assay precision, twenty measurements of the low and high QC standards were pulled from routine sample batches and the average and standard deviation calculated. The inter- and intra-assay precision values were better than 10%. To determine the accuracy of the LC/MS/MS method, forty samples were run using the LC/MS/MS method and the validated GC/MS method. The two sets of data showed good agreement, with a linear correlation of 0.90. Transferring the opiate method from GC/MS to LC/MS/MS showed improvements in detection limits of at least 5x, as well as great time and cost savings from the simplified sample preparation and reduction in number of samples that required re-analysis.

Keywords: **LC/MS/MS, Opiates, Fentanyl**

[1] Dahn, T., Shanks, K., and Sasaki, T. A.; SOFT Meeting, Austin, TX, 2006, Poster P27.

P43 Simultaneous GC/EI-MS Determination of Δ^9-Tetrahydrocannabinol, 11-Hydroxy-Δ^9-tetrahydrocannabinol, and 11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol in Human Urine Following Tandem Enzyme – Alkaline Hydrolysis

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A sensitive and specific GC/EI-MS method was modified and validated for the simultaneous extraction and quantification of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) in human urine. THC and its hydroxylated and carboxylated metabolites are conjugated prior to urinary excretion. Gas chromatography/mass spectrometry (GC/MS) methods require hydrolysis of urine conjugates prior to extraction and analysis to capture total analyte concentration.

To ensure complete hydrolysis of conjugates and capture of total analyte content, urine samples were hydrolyzed by two methods in series. Initial hydrolysis was with *Escherichia coli* β-glucuronidase (Type IX–A). Two mL urine fortified with THC-d_3, 11-OH-THC-d_3 and THCCOOH-d_3 was hydrolyzed with 0.5 mL of 20,000 units/mL of *E. coli* β-glucuronidase (final conc. 5000 u/mL) for 16 h at 37^{o}C in a shaking water bath followed by a second hydrolysis utilizing 80µL of 10N NaOH at 60^{o}C for 20 min. Specimens were adjusted to pH 5-6.5 with 50 µL concentrated glacial acetic acid. Two mL of acetonitrile were added to precipitate protein followed by 2 mL 2N sodium acetate buffer (pH 4.0). Specimens were centrifuged and supernatants applied to conditioned solid phase extraction (SPE) columns. SPE columns were washed with 3 mL deionized water, 2 mL 0.1N hydrochloric acid /acetonitrile (70:30 v/v), and dried by full vacuum for 10 min. After priming the sorbent bed with 0.2 mL hexane, analytes were eluted with 5 mL elution solvent (hexane:ethyl acetate 80:20 v/v) into tubes containing 0.5 mL ethanol. Extracts were reconstituted with 25 µL acetonitrile, transferred to autosampler vials, and 20 µL BSTFA was added. Vials were capped and derivatized at 85°C for 30 min. Extracted analytes were simultaneously quantified by gas chromatography–mass spectrometry with electron impact ionization (GC/EI-MS).

THC and 11-OH-THC were recovered by *E.coli* β-glucuronidase hydrolysis but not by alkaline hydrolysis or when no hydrolysis was performed. Alkaline hydrolysis with 10 N NaOH provided more effective recovery of THCCOOH than enzyme, and the tandem enzyme-alkaline hydrolysis procedure demonstrated the most complete recovery of analytes. Combining enzyme and alkaline hydrolysis in sequence enabled efficient recovery of THC, 11-OH-THC, and THCCOOH conjugates. The three analytes were quantified simultaneously with dynamic ranges of 2.5 to 300 ng/mL. Extraction efficiencies were 57.0 - 59.3% for THC, 68.3 - 75.5% for 11-OH-THC, and 71.5 - 79.7% for THCCOOH. Intra and inter-assay precision across the linear range of the assay ranged from 0.1 - 4.3% and 2.6 - 7.4%, respectively. Accuracy was within 15% of target concentrations. This method was applied to the analysis of urine specimens collected from individuals participating in controlled and withdrawal cannabis administration studies, and may be a useful analytical procedure for determining recency of cannabis use in forensic toxicology applications.

Keywords: **Tetrahydrocannabinol, GC/MS, Urine, Hydrolysis**

P44 The Analysis for Δ^9-Tetrahydrocannabinol and 11-Nor-9- Carboxy-Δ^9-Tetrahydrocannabinol in Plasma by Liquid Chromatography-Quadrupole-Time of Flight Mass Spectrometry

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Because marihuana impairs driving and other complex tasks, the analysis for Δ^9-tetrahydrocannabinol (THC) and its major metabolite 11-Nor-9-carboxy-THC (THCA) in plasma and blood continues to be essential. A method that was used to measure THC and THCA in oral fluid samples by liquid chromatography-quadrupole-time of flight mass spectrometry (LC-QTOF) (1) was adapted to analyze plasma samples. The method utilized silanized glassware, a 1 mL specimen volume and THC-d_3 / THCA-d_3 internal standards. Assay preparation involved an acetonitrile precipitation step and a two step liquid-liquid extraction that used hexane : ethyl acetate (9:1) as the extraction solvent. The first extraction was under basic conditions and recovered the THC. The second extraction was under acidic conditions and recovered the THCA. The extracts were combined, evaporated, reconstituted in mobile phase, and transferred to autosampler vials. The LC-MS-MS system was an Agilent (Palo Alto, CA) 1100 HPLC coupled with an Applied Biosystems (Toronto, Ontario) QTOF MS. A Waters (Milford, MA) Xterra C_{18}, 3.5 µm, 2.1 X 150 mm LC column was used. The mobile phase was a gradient that used 10 mM ammonium formate, pH 3.5 (solvent A) and methanol (solvent B). The initial conditions were 23 % A and 77 % B. After 0.2 minutes, the conditions were ramped to 4 % A, 96 % B and held for 3 minutes. Then, the gradient was returned to 23 % A, 77 % B over 1.5 minutes and held for 11.5 minutes. The flow rate was 0.2 mL/minutes. Under these conditions, the THCA eluted at 7.9 minutes and the THC at 9.6 minutes. The MS used a TurboIon Spray electrospray ion source in the positive ion mode. The scan mode used was product ion. The precursor and product ions for the compounds were – THC: 315.2 (193, 259); THC-d_3: 318.2 (196, 262); THCA : 345.2 (299, 327); THCA-d_3 : 348.2 (302, 330). The range for quantitation was 0.5 to 100 ng/mL for THC and 1.0 to 100 ng/mL for THCA. Quality control samples were prepared at 1.5 ng/mL, 10 ng/mL and 80 ng/mL. From 4 different analytical runs, the inter-assay accuracy evaluation showed that the THC and THCA concentrations in the controls were within 8% of the target. For the inter-assay precision, the coefficients of variation ranged from 3.8 to 13.8%. The high resolving character of TOF MS permits a high degree of specificity for the analysis of THC and THCA. This method also has the advantage of not needing derivatization compared to traditional GC-MS analysis.

1. Quintela, O., Andrenyak, D.M., Hoggan, A.M., Crouch, D.J. A validated method for the detection of Δ^9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol in oral fluid samples by liquid chromatography coupled with quadrupole-time-of- flight mass spectrometry. Journal of Analytical Toxicology **31**: 157-164 (2007).

Keywords: **LC/QTOF, Tetrahydrocannabinol, Plasma**

P45 An ONLINE DAT®*** Immunoassay for the Detection of Amphetamine, Methamphetamine and Designer Amphetamines in Urine

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A new assay is in development for the detection of amphetamine and related compounds in urine on automated clinical analyzers**. The liquid, homogeneous assays utilize the KIMS technology (Kinetic Interaction of Microparticles in Solution), where multiple monoclonal antibodies are covalently linked to carboxy-modified polystyrene microparticles, with drug conjugates in solution. The assay utilizes cutoff concentrations of 300, 500 and 1000 ng/mL. Studies summarized herein evaluate the 500 ng/mL cutoff (comparable results obtained for the 300 and 1000 ng/mL cutoff). When run in semi-quantitative mode on Roche/Hitachi 917 analyzers, control recovery at 250, 375, 500, 625 and 1000 ng/mL showed a recovery of 266 (106%), 387 ng/mL (103%), 511 ng/mL (102%), 648 ng/mL (104%) and 1042 (104%) with intra-assay %CV precision values of 3.6%, 3.1%, 3.2%, 2.9% and 2.9%, respectively. The interassay precision of the same levels ranged from 3.3 to 4.4%. High cross-reactivity to designer drugs is observed (d-Methamphetamine is ≥ 90%, d-Amphetamine is 75-125%, BDB is ≥ 30% and MBDB, MDEA, MDA and MDMA are ≥ 50%). Cross-reactivity to over-the-counter medications such as l-ephedrine and d-pseudoephedrine is ≤ 1%. Studies show the immunoassay displays a low background and the screening of samples that challenge the cutoff all give the correct response. An internal method comparison versus samples analyzed via GC/MS (50 positive samples and 10 negative samples n=10) demonstrated 100% correlation. GC/MS confirmed negative clinical samples give an average reading of 39.7 ng/mL. The above studies demonstrate that this assay provides an accurate and precise method for screening urine for the detection of amphetamines and related compounds on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.
*** ONLINE DAT is a trademark of Roche.

Keywords: **Amphetamine, Methamphetamine, Immunoassay**

P46 A Validated Gas Chromatographic – Electron Impact Ionization Mass Spectrometric Method for Methylenedioxymethamphetamine, Methamphetamine and Their Metabolites in Mouse Plasma

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Background: Human and animal studies indicate long-term exposure to methamphetamine (MAMP), amphetamine (AMP), or methylenedioxymethamphetamine (MDMA) is neurotoxic, causing decreases in neurotransmitter concentrations and neurotransmitter transport proteins. P-glycoprotein (Pgp) is a drug efflux transport protein that resides in the blood-brain barrier and limits neurotoxicity by actively transporting drugs that reach the brain back out into blood. Preliminary studies using Pgp knock-out mice that lack Pgp protein, demonstrated that Pgp limited MAMP neurotoxicity. Surprisingly, these studies revealed that Pgp potentiated MDMA neurotoxicity. These preliminary studies did not measure blood or brain concentrations of MDMA, MAMP and metabolites to establish if there is a direct relationship between METH and MDMA concentrations in the brain and levels of toxicity observed in Pgp knock-out mice. The objective of this study was to develop and validate an assay that will be employed for measuring MDMA, MAMP and metabolites in plasma from wild-type and Pgp knock-out mice collected during studies investigating the role of Pgp in MDMA and MAMP neurotoxicity.

Methods: Sample volume collected from mice during our studies is limited, typically less than 200 microliters. Therefore, it was necessary to develop a sensitive assay capable of measuring MAMP, AMP, hydroxy-methamphetamine (OH-MAMP), MDMA, methylenedioxyamphetamine (MDA), hydroxy-methoxymethamphetamine (HMMA) and hydroxy-methoxyamphetamine (HMA) in 100 microliters of plasma. Calibrators and internal standards were added to 100 microliters of plasma. Samples underwent protein precipitation using ice-cold trichloroacetic acid. Supernatants were collected in screw-top reacti-vials, 100 microliters of 12M hydrochloric acid was added and the vials were capped tightly. Glucuronidated metabolites of MDMA were acid hydrolyzed at 100°C for 45 minutes. After cooling, samples were neutralized with 6M sodium hydroxide and diluted with 0.2M acetate buffer, pH 4.5. Prepared samples were extracted using SPEC MP1 solid phase extraction columns. Ethyl acetate: methanol: ammonium hydroxide (77:20:3 v/v/v) was utilized to elute the analytes of interest. Dried extracts were derivatized with heptafluorobutyric acid and analyzed on an Agilent 6890 gas chromatograph with an Agilent 5975 mass selective detector operated in electron impact ionization selected ion monitoring mode.

Results: Limits of detection were 2.5 ng/mL for MAMP, OH-MAMP, HMMA and HMA and 5.0 ng/mL for AMP, MDA and MDMA. Linear dynamic ranges were 10-1000 ng/mL for MAMP, AMP, MDMA and MDA and 20-1000 ng/mL for OH-MAMP, HMMA and HMA. Analyte recoveries were greater than 91%. Interassay accuracy and precision ranged between 91-113 % of target concentration with 1.5 – 6.6% coefficient of variation, respectively (n=14).

Conclusion: This assay for measuring MAMP, MDMA and metabolites in mouse plasma will be useful for studies investigating the role of Pgp drug efflux in MAMP and MDMA neurotoxicity.

Keywords: **Methamphetamine, MDMA, Neurotoxicity**

P47 Analysis of MTPA Derivatized Methamphetamine by GC/MS – An Alternate Ion

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Enantiomer analysis of methamphetamine has been accomplished using a variety of different methods over the past decades. Knowing the enantiomeric distribution of methamphetamine, and its metabolite amphetamine, is critically important to properly interpret the analytical data. Indirect determination of the enantiomers by derivatization using a chiral derivatizing reagent is a common procedure. In these cases the determination of the enantiomers is based on chromatographic determination of the diasteriomer formed by derivatization of the drug with a chiral derivatizing reagent. Determination of methamphetamine enantiomers is commonly accomplished as a two step process. The first being a quantitative, and qualitative, analysis of methamphetamine derivatized using an achiral reagent such as HFBA. In this case the compound is identified by meeting accepted criteria for identification of a compound (i.e. three symmetrical ions at a specific retention time demonstrating ratios demonstrative of the analyte (within acceptable analytical variation, etc.). The assay also allows quantitation but not determination of the enantiomers since they co-elute under those analytical conditions. Derivatization using a chiral reagent allows physical separation of the enantiomers and determination of the proportion of each enantiomer. There are a few examples of assays that proposed both qualitative, quantitative and enantiomer analysis within the same assay but these have not been widely used within the community. Such procedures do offer the ability to get all of the answers with a single assay rather that multiple assays. One of the proposed methods used MTPA as the derivatizing reagent.

The current study evaluates the utility of using an ion at m/z 200 for identification of the drug. The initial method suggested the use of ions at m/z 176, 274 and 275. The SOFT-AAFS Guidelines specifically describes the use of isotope ions as unacceptable for the qualitative identification of compounds by MS analysis. Use of the ion at m/z 200 overcomes that weakness and allows identification of the compound meeting all acceptance criteria. The ion proved to produce consistent, acceptable ion chromatography comparable to that for the other monitored ions and was stable across analytical runs staying well within the +/-20% ion ratio acceptance criteria. Analysis of several hundred samples from controls and drug users showed no failures caused by the m/z 200 ion itself.

Keywords: **Methamphetamine, Enantiomers, MTPA**

P48 Chiral Separation of Cathinone, Metabolites and Related Substances using Gas Chromatography

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Catha Edulis or Khat is a central nervous stimulant scheduled as an illicit drug in Sweden. The main active alkaloid of Khat is (-)-cathinone together with the less abundant (+)-norpseudoephedrine (cathine) both of which are scheduled substances and (-)-norephedrine which is not scheduled. Norephedrine, ephedrine and pseudoephedrine are also found in non-scheduled pharmaceuticals. Thus, if no cathinone is present in the sample a chiral analysis distinguishing between enantiomers has to be performed. The aim of this work was to study different analytical approaches for enantiomeric separation of cathinone, norephedrine and structurally related substances in urine, with the purpose to find a method that unequivocally could prove Khat intake. Besides (-)-cathinone, (+)-cathine and the metabolite (-)-norephedrine the study also included (+)-cathinone, (+)-norephedrine, (-)-pseudoephedrine, (+)-pseudoephedrine, (-)-ephedrine and (+)-ephedrine. Reference material for the metabolite (-)-cathine was not available.

Two different methodologies were tested and compared: (i) a chiral reagent forming diastereomers and subsequent separation on an achiral stationary phase or (ii) chiral column chromatography of different derivatives of achiral reagents (or underivatized). Possible interferences from intake of the medication Disofrol, containing (+)-pseudoephedrine, were investigated by analysis of urine samples collected after a single dose of Disofrol. In addition, seven authentic urine samples were analyzed and the substances were quantified using (+)-ephedrine-d_3 as internal standard. After solid phase extraction on Bond Elut Certify cartridges and derivatization, the samples were analyzed by GC-MS.

The GC-MS analysis was performed on a Hewlett Packard (HP) 6890 gas chromatograph with an HP 5973 mass selective detector and equipped with a CTC autosampler. The derivatives of the chiral reagent S-(-)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) were analyzed using an HP-5MS 5% phenylmethylsiloxane column, 30 m x 0.25 mm x 0.25 μm. The chiral column chromatography were carried out on two beta-cyclodextrin (CD) columns, 30 m x 0.25 mm x 0.25 μm, one coated with 2,3-di-O-methyl-6-O-tert-butyldimethylsilyl-beta-CD (Rt-βDEXsm™) and one beta-CD (Rt-βDEXcst™) with undeclared stationary phase.

The nine compounds were successfully resolved using the chiral reagent MTPA. The chiral column Rt-βDEXcst™ showed good separation for all compounds as TFAA-derivatives except for the enantiomeric pairs of ephedrine. No separation was achieved for TFAA-derivatives using the chiral column Rt-βDEXsm™. Underivatized compounds were not resolved at all on either of the chiral columns. All urine samples collected after intake of Disofrol contained high levels of (+)-pseudoephedrine together with small amounts of the metabolite (+)-cathine. For the seven samples that were found positive for Khat (-)-norephedrine and (+)-cathine were measured in the range of 15-88 μg/mL urine and (-)-cathinone and (+)-cathinone in the range of 0.1-8.6 μg/mL urine.

In conclusion, among the tested approaches, chiral derivatization by MTPA and chiral column chromatography by Rt-βDEXcst™ of TFAA-derivatives proved to be suitable for enantiomeric separation and identification of Khat intake.

Keywords: **Khat, Chiral, GC/MS**

P49 Comprehensive Analysis of Drugs and Metabolites in Urine with Automated Disposable Pipette Extraction

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The analysis of basic, acidic and neutral drugs in urine is accomplished with a single small volume sample solution using two types of disposable pipette extraction (DPX) products. One type of DPX tip (DPX-RP) is optimal for extracting acidic and neutral drugs and even several basic drugs using reverse phase and hydrophobic mechanisms. The urine sample solution is re-extracted using a second DPX tip (DPX-CX) that incorporates cation exchange mechanisms to recover essentially all basic drugs.

The recoveries of the acidic and neutral drugs, such as barbiturates, glutethimide, and COOH-THC, are greater than 80% using DPX-RP. Also, several basic drugs such as several tricyclic antidepressants, PCP, and meperidine, are also extracted with these high efficiencies. Using DPX-CX tips, other basic drugs such as opiates, amphetamine and methamphetamine are extracted with efficiencies between 80 to 100%. Combining the two types of extraction methods, comprehensive screening is readily accomplished with a single small volume of sample (200uL).

The main advantages of DPX technology is that the extractions are very rapid, negligible solvent waste is generated, and the extractions can be fully automated and coupled to chromatographic injections. Using a Gerstel MPS-2 instrument, the DPX extractions can be performed off-line or “in-line” with a GC or HPLC instrument. This study includes both applications of automated DPX extractions. It is found that the extractions can be performed during the time required for chromatographic analysis, and therefore throughput can be optimized by performing the analyses “in-line” with chromatographic instrumentation. In this study, the comprehensive analysis of just 0.2 mL of urine samples are preformed “in-line” with GC/MS (Agilent Technologies 6890 with 5973 MSD) with run times of less than 10 minutes each.

The advantages of full automation are discussed, with emphasis on chain-of-custody and tracking of evidence. The automation minimizes possible sample handling errors as well as technical training.

Keywords: **Solid Phase Extraction, Disposable Pipette Extraction, GC/MS**

P50 A Semi-Quantitative General Unknown Screening Method for Drugs and Toxic Compounds in Urine Using Liquid Chromatography–Mass Spectrometry

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Introduction and Objective: Many clinical and forensics laboratories utilize general unknown screening (GUS) to identify analytes present in biological samples. Often, once an analyte is identified, laboratories use a second method for quantitation. The goal of this work was to develop one method on a triple quadrupole LC-MS system that could be used to both identify unknown analytes in human urine and determine their concentration semi-quantitatively. Clinicians under time constraints to screen samples such as those in an emergency room setting, could potentially benefit from such a method.

Method: An MS/MS spectral library of 300 compounds most commonly observed in toxicology laboratories was created. Quantitation curves were developed for 50 analytes in SPE prepped human urine. Three deuterated internal standards (100 µL from a 1 µg/mL solution) were added to each sample (1 mL of urine) prior to SPE, for quality control. The SPE method utilized dual mode Hypersep-Verify CX cartridges. The basic fraction was eluted with 1.5 mL of ethyl acetate/NH_4OH 98/2 (v/v) and 1.5 mL of CH_2Cl_2/IPA/NH_4OH 78/20/2 (v/v/v). Acidic and neutral fractions were eluted with 1.5 mL of acetone/chloroform 50/50 (v/v) and 1.5 mL acetone/CH_2Cl_2 50/50 (v/v). A 13 minute LC method was implemented and samples were analyzed using electrospray ionization on a TSQ Quantum Access triple quadrupole mass spectrometer. A scan dependent SRM scan was used for quantitation, followed by scan dependent MS/MS scans for screening against the spectral library.

Results: The screening of 300 compounds was validated by processing and analyzing urine samples spiked with 10 randomly selected compounds in concentrations of 1ng/ml, 10 ng/mL, 100 ng/mL, 500ng/mL and 1000 ng/mL for all 300 analytes. The recoveries from SPE were estimated for all compounds. Calibration curves for 50 compounds were obtained from SPE spiked urine samples and LOQ's were reported for the 50 analytes. All 300 compounds from the library were analyzed. Approximately 90% were detected in concentrations ranging form 1-1000 ng/mL of urine. The method was confirmed in patient urine samples and compared to an established GUS method on an LXQ ion trap mass spectrometer.

Conclusion: Results show that the LC-MS based GUS method developed on a triple quadrupole mass spectrometer can properly identify analytes in urine at concentrations ranging from 1-1000 ng/mL, with automated report generation. In addition, for at least 50 compounds, concentrations can be semi-quantitatively determined.

Keywords: **General Unknown Screening, Mass Spectrometry, Triple Quadrupole**

P51 Rapid Multidimensional GC Analysis of Trace Drugs in Complex Matrices

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Reduction in analysis time is an important goal for easing the burden of large sample sets associated with routine screening of blood samples and sample sets produced from drug metabolism studies.

This study focuses on reduction in analysis time for simultaneous detection of delta-9-THC, 11-OH-THC, and THC-COOH in whole blood. This was achieved using the GERSTEL MACH fast GC system combined with an Agilent GC-MSD. The addition of the MACH system allows three independently heated temperature zones for multidimensional chromatography using the Agilent Capillary Flow Technology Deans Switch along with fast heating/cooling rates. A novel pre-column approach protects the analytical columns in the independent temperature zones and adds a high level of robustness. The Agilent Capillary Flow Technology Dean's Switch allows a combination of heart-cutting multidimensional GC and backflushing to reduce the amount of unwanted background components.

Standard analysis time was reduced from 18 minutes to under 12 minutes. The linearity of the three analytes was from 0.35 ng/ml to 50 ng/ml . Two Agilent Capillary Flow Technology Deans Switches in tandem were used for this analysis. Three independently programmed pressure zones were used in conjunction with three independent heated zones. The MS was operated in the EI mode.

Keywords**: Drug Analysis, Blood, Fast GC/MS**

P52 The Use of Accurate Mass, High Resolution, and Isotope Ratios in an Algorithm Applied to Time-of-Flight Mass Spectrometric Data for the Identification of Drug Compounds in Blood

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Objective: An algorithm known as Molecular Feature Extractor is developed and applied to full scan mass spectral data acquired on a time-of-flight mass spectrometer (TOF). The spectral data acquired on a TOF that has a performance specification of less than 2 ppm mass accuracy and resolution up to 10,000, is investigated for the presence of molecular features, which have certain isotopic and adduct-ion patterns. These features are then matched to a database of known compound empirical formula. However, as the database is further developed, future inspection of the same data can be carried out to look for additional compounds that weren't being investigated before.

Methods: Sample preparation involves a simple protein precipitation step followed by solid phase extraction. As this is an LC/MS analysis no derivatization of the sample is required. Following atmospheric pressure ionization, accurate mass and high resolution spectral data is acquired and then investigated by a special algorithm for the determination of features which may or may not correspond to known drug compounds in a database.

Results: Of the many features derived from the acquired data, the following table demonstrates the results of analyzing a blood sample for drug content. For the sample below, some insecticides also present.

Measured Mass	Formula	Compound	Exact Mass	Error (mDa)	Error (ppm)	Description
266.16274	C14H22N2O3	Atenolol	266.163	-0.3	-1.1	Beta-Blocker
194.08078	C8H10N4O2	Caffeine	194.0804	0.4	2.1	Stimulant
201.07963	C12H11NO2	Carbaryl	201.079	0.65	3.2	Insecticide
221.10511	C12H15NO3	Carbofuran	221.1052	-0.08	-0.4	Insecticide
274.12364	C16H19N2Cl	Chlorpheniramine	274.1237	-0.04	-0.1	Antihistamine
165.1152	C10H15NO	Ephedrine	165.1154	-0.16	-1.0	Sympathomimetic
330.03606	C10H19O6PS2	Malathion	330.0361	-0.01	0.0	Insecticide
246.10086	C13H14N2O3	Methylphenobarbital	246.1004	0.42	1.7	Hypnotic
151.06334	C8H9NO2	Paracetamol	151.0633	0.01	0.1	Analgesic
209.10557	C11H15NO3	Propoxur	209.1052	0.38	1.8	Insecticide
165.1152	C10H15NO	Pseudoephedrine	165.1154	-0.16	-1.0	Bronchodilator
277.20399	C17H27NO2	Venlafaxine	277.2042	-0.19	-0.7	Antidepressant

Conclusion: Several blood samples are successfully analyzed by LC/MS using a TOF instrument. The power of accurate mass is demonstrated in identifying compounds using empirical formula. High resolution in the mass spectrum allows the determination of co-eluting compounds and isotopic patterns for each compound. While the Molecular Feature Extractor finds a large number of possible compounds in any given sample, a database is successfully referenced to identify and confirm the presence of a select few of them. However, the full scan mass spectral data still exists for future determination of compounds that may be added to the database later. The primary purpose of the algorithm is to save the user time in analyzing large amounts of data.

Keywords: **Time Of Flight Mass Spectrometry, Accurate Mass, Drug Analysis**

P53 A Novel, Turn-Key LC/MS/MS Replacement Strategy for Traditional LC/UV Drug Screening Technology

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The ratio of mass spectrometry experts to non-experts has been declining over the years, as LC/MS/MS finds new applications over a broad range of markets, such as forensics, toxicology, food testing, environmental analysis, etc. An obvious response to this problem is to reduce the complexity level of the user interface to the system, and thereby to reduce the expertise level required by the operator. Such a solution works only if the acquisition and processing know-how is built into the system.

An example of an application where LC/MS/MS is set to displace a traditional technique is forensic drug screening. For the last 20 years, the technique of choice for rapid screening has been LC/UV with automated on-line sample preparation, and reporting, which reduces the analysis to a very simple operation. Replacing this type of technique with LC/MS/MS presents a challenge in the design of the software and development of the acquisition and processing methods.

A challenge in the development of LC/MS/MS methods for drug screening is the creation of chromatographic separation and mass spectrometric detection methods to cover the broad range of applicable compounds. By combining extraction techniques with a chromatographic separation on reversed phase material, and mass spectrometric detection in Multiple Reaction Monitoring (MRM) followed by automatically triggered Enhanced Product Ion (EPI) scanning and library searching on a hybrid triple quadrupole linear ion trap instrument (Q TRAP® LC/MS/MS system) a methodology and workflow has been developed that provides superior performance over the existing state-of-the-art LC/UV, without adding complexity to the screening procedure. This paper presents the results of a development project to create a turn-key replacement LC/MS/MS solution for drug screening. The system performance characteristics and examples are provided, along with a back-to-back comparison with traditional LC/UV techniques. Cliquid™ Software provided an ideal platform for development of an easy-to-use turn-key solution enabling the selective and sensitive screening for hundreds of drugs of abuse, pharmaceuticals and their metabolites followed by library searching to confirm the presence of identified compounds.

Keywords: **LC/MS/MS, Screening, Library Searching**

P54 General Unknown Multi Target Screening (MTS) using a QTrap with ESI-MS/MS Library of 1250 Compounds

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Objective: Development of a preliminary MTS screening procedure to its routine use for general unknown screening.

Methods: A library with ESI MS/MS spectra of 1250 compounds has been developed using a QTrap 3200 tandem-mass spectrometer (Applied Biosystems) with a turboionspray source.
After standardization of a chromatographic system using a 50 mm x 2.1 mm Allure PFP column (Restek), the library has been used for the identification of drugs and metabolites in urine and serum samples using a "multi target" general-unknown screening approach. Retention times of 800 compounds have been determined and transitions for each compound were selected by a survey-MRM scan, followed by an information dependent acquisition (IDA) using the sensitive enhanced product ion scan of the Qtrap hybrid instrument. A library search was performed for compound identification. Due to the selection of MRM transitions, the method is called Multi Target Screening, covering more than 800 compounds (drugs of abuse, psychoactive drugs and many others).

Results: Standardization of the procedure has been performed for its applicability in different laboratories, using a reference standard test mixture ("MTS Tuning Mix"), and also internal deuterated standards for semiquantitative analysis for several drugs. First applications of this procedure have been developed for the detection and identification of drugs of abuse and drugs for substitution (opiates, amphetamines, cocaine, LSD, cannabinoids, buprenorphine, methadone) and psychopharmaceuticals (benzodiazepines, hypnotics, antidepressants, neuroleptics) and pain relief drugs. Urine samples of drug abusers and clinical cases have been investigated, with the aim of testing the reproducibility and robustness of the system, especially in terms of comparison of different sample preparation procedures (dilution, extraction) and matrix effects.
With the use of the internal standards, the system could be used for drug identification and simultaneously for semiquantitative confirmatory analysis – as will be demonstrated by GC/MS and HPLC-DAD analysis performed in parallel. Due to an optimized column and gradient for elution with steadily increasing flow rate at the end of the analytical run, a great variety of compounds was detectable in a retention time window of 17 mins – in many cases, even at lower concentrations than detectable by classical immunoassays. Human plasma was spiked with several compounds including MDMA, carbamazepine, diphenhydramine, tramadol, melperone, doxepine, sertraline and LSD, which could be identified at 1 ng/mL after liquid-liquid-extraction with chlorobutane at pH 9.

Conclusions: The application of this screening method is in clinical toxicology (for target analysis in intoxication cases), in psychiatry (antidepressants and other psychoactive drugs), in forensic toxicology (drugs and driving, workplace drug testing, oral fluid analysis, drug facilitated sexual assault) – whenever a huge number of different drugs are relevant. Further challenges are the automation by on-line extraction for plasma or oral fluid samples and by reporting by Cliquid software, a tool for method set-up, data acquisition and reporting - supplied by the instrument manufacturer.

Keywords: **ESI-MS/MS, General Unknown Screening, MS/MS Library**

P55 Development of an LC/MS/MS System Suitability Test

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Objective: When evaluating the overall performance of an LC/MS/MS system, it is necessary to design a test that will assess each component: LC pump, autosampler, column, and mass spectrometer. Selected compounds should span a large polarity, therefore retention time, range and the compounds should also cover the mass range of interest. If full scan MS/MS is utilized, the fragmentation capabilities of the instrument must also be evaluated. The objective of this presentation is to identify the important criteria to consider when evaluating LC/MS/MS system performance and develop a suitable system check.

Methods: Systems consisted of various LC stacks interfaced to hybrid triple quadrupole/linear ion trap (QQQ/LIT) mass spectrometers. Hybrid QQQ/LIT instruments were used so that both MRM and full scan MS/MS capabilities could be evaluated. The analytes used were caffeine, morphine, codeine, haloperidol, amiodarone, methamphetamine, doxepin and diazepam. Mobile phases were: A) 1 mM ammonium formate and B) 95:5 acetonitrile:1 mM ammonium formate with 0.1% formic acid added to each. An Applied Bisoystems pentafluorophenyl column (2.1 mm x 50 mm) was used for separation and a rapid gradient was used to elute the compounds. Total run time was 15 min. This test was designed to check the performance only in ESI+ mode. MRM transitions and their optimal collision energies for each analyte were determined and two transitions per analyte were monitored.

Results: Several analytes were used to evaluate the performance of several LC/MS/MS systems. Morphine and caffeine were chosen as a polar, relatively unretained analytes and amiodarone was used as a relatively non-polar, late eluting analyte. Haloperidol exhibited a fragmentation pattern that was useful for evaluating the fragmentation performance of the mass spectrometer. Inter- and intra-day reproducibility of retention times, peak intensities, and peak area ratios were recorded and monitored to gauge instrument performance over time. A change in any of these parameters, such as peak shape or intensity, could indicate hardware problems, e.g. pumping problems or a dirty instrument.

Conclusion: As LC/MS/MS is increasingly used in routine forensic analyses, a quick, effective system suitability test is necessary to evaluate the system performance and troubleshoot any potential problems. By using a test mixture that includes analytes over wide polarity and mass ranges, system performance can be checked and monitored to identify any problems before case samples are run.

Keywords: **Mass Spectrometer, LC/MS/MS, Drug Analysis**

P56 A Novel Approach to Evaluate the Extent of Cross-Contribution to the Intensity of Ions Designating the Analyte and the Internal Standard in Quantitative GC-MS Analysis

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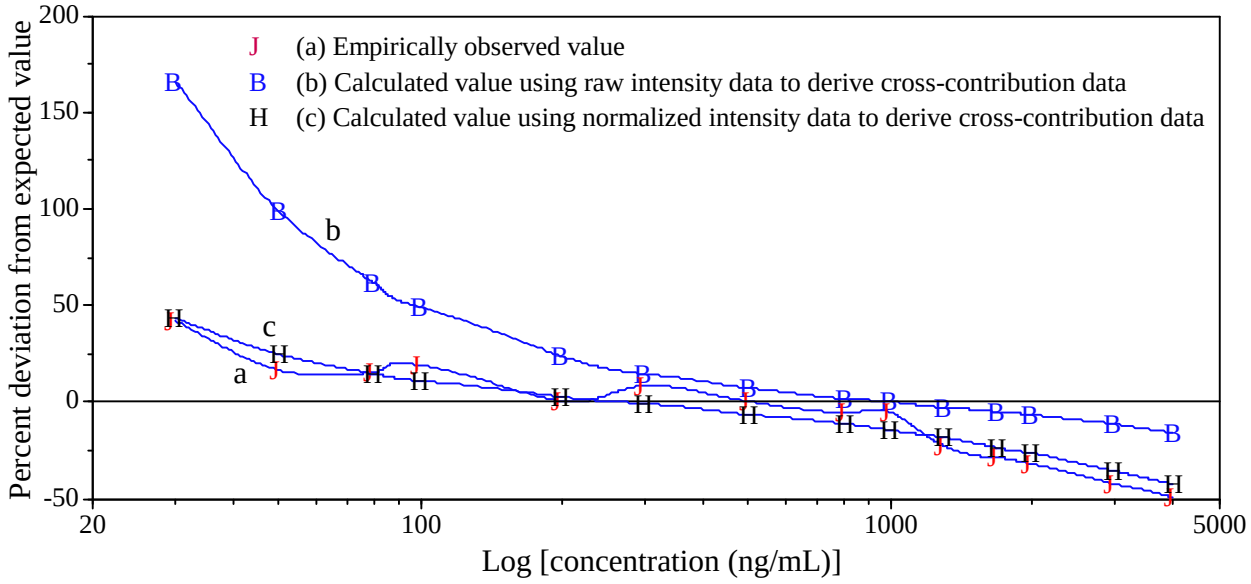
Gas chromatography-mass spectrometry (GC-MS) methods adapting isotopically labeled analogs of the analytes as the internal standards (ISs) are routinely used and universally considered the most effective approaches for the analysis of drugs/metabolites in biological specimens. One important factor that may affect the accuracy of the quanitative data is the intensity cross-contribution (CC) between the analyte and the IS, i.e., the contribution of IS to the intensities of the ions designating the analyte, and vice versa. Thus, it is imperative to know the CC values between the ions designating the analyte and the isotopic analog before adapting the latter as the IS. Mathematic models can then be applied to correct errors that may derive form the CC phenomenon.

We have reported several approaches for the calculation of the CC values for any analyte/isotopic analog pair. This study was conducted to develop a novel approach, based on the deviations of the empirically observed concentrations of a set of standards from their true values, to assess the accuracy of the empirically determined CC data. Adapting 3,4-methylenedioxyamphetamine (MDA)/MDA-d_5 and hydromorphone/hydro-morphone-d_6 as the exemplar analyte/IS pairs, a typical set of data derived from *m/z* 100/104 (designating MDA/MDA-d_5) are plotted in the figure shown below. Empirically determined CC data were used to first theoretically calculate the expected concentrations of these standards. Their derivations from the true concentrations of these standards are plotted in curve "b". This curve is significantly different from the curve ("a") that was plotted using empirically observed concentrations. This is an indication that the empirically determined CC data are not accurate. Curve "c" was the result when the empirically determined CC data were first corrected and then used for the theoretical calculation. The "closeness" of curves "a" and "c" indicates the corrected set of DD data is accurate.

The theoretical calculation model was further used to demonstrate that an ion-pair (designating the analyte and the IS) with approximately 5% or higher CC, when used for quantitation, will result in a very limited linear calibration range.



Keywords: **Quantitative Analysis, Internal Standard, GC/MS**

P57 The Certification of Drugs of Abuse in a Human Serum Standard Reference Material

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Drug abuse is a significant problem worldwide. The determination of drugs of abuse in seized contraband, or within bodily fluids, acquired for routine screening or autopsy purposes, is one of the most common types of analyses carried out in forensic laboratories. The consequences of a positive test can be quite severe and can be cause for dismissal from the workplace or may lead to further legal action. As a result, laboratories conducting such tests must rigorously follow a carefully designed quality assurance program.

Currently NIST supports accuracy in drugs of abuse testing by providing Standard Reference Materials (SRMs) with certified concentrations of drugs of abuse in urine- and hair-based reference materials. Many jurisdictions, however, allow urine or blood to be sampled, requiring laboratories to have valid procedures for either bodily fluid. Blood is often regarded as the preferred matrix as it is less susceptible to adulteration and directly correlates to the degree of impairment in an individual. This new SRM material will act as a control material and assure long term integrity and accuracy in the methods used to detect and quantify drugs of abuse in blood.

This project involved the preparation and certification of SRM 1959 Drugs of Abuse in Human Serum. The material was prepared by spiking the serum matrix with 7 different drugs of abuse: benzoylecgonine, methadone, methamphetamine, morphine, nordiazepam, phencyclidine, and (±)-11-Nor-9-carboxy-Δ9-tetrahydrocannabinol, at a concentration of approximately 1 µg/mL. Extraction methods were developed and optimized for the detection of the target analytes. Sealed ampoules of the selected drugs in the frozen serum matrix were defrosted, analyzed and subjected to the certification process. Certified concentrations of each analyte were obtained from the concordant results by two independent analytical methods. The first method involved extraction using mixed mode Solid Phase Extraction (SPE) cartridges, derivatization, and analysis by isotope dilution gas chromatography / mass spectrometry (GC/MS). The second method involved extraction using reverse phase SPE columns, and analysis by isotope dilution liquid chromatography / mass spectrometry (LC/MS). The results from the two methods were in good agreement with a mean difference for the analytes ranging from 0.6 to 8.1 %. This SRM can assist laboratories in evaluating the accuracy and precision of their methods in both clinical and forensic toxicology.

Keywords: **Drugs of Abuse, Serum, Chromatography / Mass Spectrometry, Quality Control**

P58 Evaluation of Microgenics DRI High Sensitivity EtG Immunoassay

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Detection of ethanol abuse is a significant problem for drug treatment facilities. Detection times for urinary ethanol after cessation of drinking are relatively short. One of the biomarkers of ethanol abuse is ethyl glucuronide (EtG). EtG can be detected for significantly longer periods of time than ethanol.

Microgenics Corporation has developed a high sensitivity EtG homogeneous enzyme immunoassay for urine testing. The EtG immunoassay was tested on a Microgenics MGC 240 Analyzer. The assay required only 30 microliters of urine. Microgenics has offered the assay as either a qualitative or semi-quantitative application. The kit included spiked calibrators from 100 to 2,000 ng/mL. To validate the assay, positive and negative controls representing 25 % above and below the 500 ng/mL cutoff were tested. The following data was based on 30 interassay determinations. The recovery for the above cutoff control produced a mean of 632 ng/mL, a standard deviation of 26.5 and a C.V. of 4.2 %. The below cutoff control yielded a mean of 372 ng/mL, a standard deviation of 18.9 and a C.V. of 5.1 %. Carryover was assessed at an EtG level of 50,000 ng/mL and none was detected. Linearity of the assay was up to 2,000 ng/mL. Cross-reactivities of a number of different abused drugs at elevated levels were studied and none was detected.

Evaluation of this DRI EtG assay was made with urine specimens from drug treatment patients and included LC-MS/MS confirmation of tests positive and negative for EtG. Twenty drug treatment patients' urine specimens were screened with the high sensitivity EtG immunoassay and analyzed on a Finnigan TSQ 7000 LC-MS/MS system. The LC-MS/MS employed a 500 ng/mL cutoff level. Fifteen of the urine specimens tested positive on the DRI EtG assay and confirmed positive by LC-MS/MS. Whereas, five of the patients' specimens tested negative by immunoassay and LC-MS/MS. Thus, the confirmation rate was 100 %.

The DRI High Sensitivity EtG Assay identified the subjects' urinary results accurately. The validation studies of the assay produced satisfactory results on a chemistry analyzer. Therefore, small sample size, relatively short analysis time and the reliability of Microgenics EtG immunoassay combine to provide a useful screening test in the effort to treat alcohol abuse.

Keywords: **Ethyl Glucuronide, Enzyme Immunoassay, LC/MS/MS**

P59 Analysis of Ethyl Glucuronide (EtG) in Urine by LC/MS and LC/MS/MS

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The purpose of this study was to compare a method for analysis of alcohol biomarkers using two different mass spectrometry systems. Alcohol biomarkers can be used as indicators of alcohol ingestion or exposure and potentially as indicators of alcohol abuse disorder. Following alcohol consumption, alcohol biomarkers such as ethyl glucuronide (EtG), have been shown to be detected in urine for many hours after ethanol itself has been eliminated. Samples were analyzed on a liquid chromatography system coupled to a single quadrupole mass spectrometer (LC/MS, analysis of parent ion) and on a liquid chromatography system coupled to a triple quadrupole mass spectrometer (LC/MS/MS, analysis of Precursor (parent) and product ions). This study evaluated sensitivity, linearity, precision, interference and other related parameters associated with method validation to determine which technology yielded the best results. Currently there is no data available describing method evaluation of EtG by two different mass spectrometry systems.

Standards spiked with concentrations of EtG ranging from 10 - 10,000 ng/mL were prepared in mobile phase and in urine. Urine samples (n = 9) collected from subjects 24 to 72 hours after alcohol consumption were also evaluated. The LC mobile phase consisted of 5% acetonitrile with 0.1% formic acid; flow rate was set at 0.2 mL/minute. The working internal standard solution contained 550 ng/mL EtG-D5 in mobile phase. A 10 µL aliquot of standard or urine was mixed with 90 µL of internal standard solution. Sample vials were placed on each instrument with injection volume set at 10 µL. Chromatographic columns were also evaluated on both systems. The mass spectrometers were set in the ESI negative mode. For the single quad, analysis was performed using single ion reaction (SIR) of *m/z* 221 (EtG) and 226 (EtG-D5). For the triple quad, analysis was performed using multiple reaction monitoring (MRM) with *m/z* 221 as the precursor ion and *m/z* 75 as the product ion. Issues of quantitation, linear range, precision, etc. are of concern, however, both methods can produce acceptable results for many typically monitored parameters. The advantage of multi-stage mass spectrometry is apparent by examination of ion chromatograms which show potential interference that is eliminated using MS-MS analysis. A fundamental question remains regarding what is an acceptable identification of an analyte by LC-MS – Is a single ion sufficient?

Keywords: **Alcohol, Ethyl Glucuronide, LC/MS**

P60 A Novel Method For Detection And Quantification Of Nicotine And Metabolites In Human Meconium By LC-MS/MS Using Atmospheric Pressure Chemical Ionization

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Nicotine exposure in utero is associated with decreased fetal growth, alterations to lung and nervous system development, behavioral disorders and increased risk of nicotine addiction later in life. Meconium is often used to detect in utero drug exposure because of its easy and non-invasive collection and long window of detection.

Objective: A liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS/MS) method for the determination of nicotine, cotinine, trans-3'-hydroxycotinine, nornicotine and norcotinine in meconium was developed and validated.

Method: Meconium specimens spiked with analytes and deuterated internal standards were sonicated in acidic methanol. After centrifugation and supernatant evaporation, enzymatic hydrolysis with β-glucuronidase was performed overnight to cleave glucuronide conjugates of nicotine, cotinine and trans-3'-hydroxycotinine. Analytes were isolated using mixed mode solid phase extraction and analyzed by LC-APCI-MS/MS operating in multiple reaction monitoring (MRM) mode. The analytical method was validated over four days for limit of quantification, accuracy, precision, recovery, matrix effects, hydrolysis efficiency, carryover and interference.

Results: The limits of quantification were 1.25 ng/g for cotinine, trans-3'-hydroxycotinine, and norcotinine, and 5 ng/g for nornicotine and nicotine; the upper limit of quantification for all analytes was 500 ng/g. Correlation coefficients for each calibration curve were >0.99. Intra- and inter-day precision ranged from 3 to 10 % relative standard deviation (RSD) and 5 to 20 % RSD, respectively. Intra- and inter-day accuracy was greater than 76%. Recoveries were all above 61%. Validation parameters were tested at 8, 80 and 400 ng/g. Ion suppression due to matrix effect ranged from 20-70%, depending on the analyte. Hydrolysis efficiencies for glucuronide conjugates of nicotine, cotinine and trans-3'-hydroxycotinine were 90, 80, 15%, respectively; the hydrolysis procedure did not affect unbound analytes. Analyte conversion to nornicotine was detected following stronger basic and acidic hydrolysis conditions. Analyte stability was assessed under the following conditions: 24 h at room temperature, 72 h at 4°C, three -20°C freeze-thaw cycles, and 24 h in the autosampler; losses of less than 30% were observed under each condition. No analyte carryover was observed at two times the upper limit of quantification. No interference by illicit and therapeutic drugs was observed.

Conclusion: The first LC-APCI-MS/MS method for the identification and quantification of nicotine, cotinine, trans-3'-hydroxycotinine, nornicotine and norcotinine in meconium is described. The method will be used in studies to correlate meconium concentrations to neonatal outcome measurements.

Keywords: **LC-APCI-MS/MS, Nicotine, Meconium**

P61 Validation and Application of a Novel Method for the Determination of Buprenorphine, Norbuprenorphine and Their Glucuronide Conjugates in Human Meconium

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A liquid chromatography tandem mass spectrometry method for the quantification of buprenorphine (BUP), norbuprenorphine (NBUP), and their glucuronidated conjugates (BUP-Gluc, NBUP-Gluc), was developed and validated. Specimens were prepared by homogenizing 0.25 ± 0.01 g of well-mixed meconium with 1.1 M sodium acetate buffer, pH 5.0. In order to quantify conjugated analytes, specimens were analyzed with and without Glusulase® hydrolysis. After homogenization, and hydrolysis if applicable, samples were centrifuged and underwent solid phase extraction with Clean Screen® extraction columns to further purify and concentrate analytes. Eluents were analyzed via LC-APCI-MS/MS in selected reaction monitoring mode. The following transitions were monitored: BUP - 468.4 → 414.3, 326.1, deuterated BUP - 472.4 → 400.2, NBUP – 414.3 → 265.2, 340.1, and deuterated NBUP - 417.4 → 268.2, 343.2. The ion ratio of either two transition ions or the parent ion and one transition ion was monitored. Calibrators (10, 20, 40, 100, 200, 400, 1000, and 2000 ng/g) were used to determine assay linearity and low, medium and high controls containing 30, 300, and 1600 ng/g free drug, 41.3, 412.5 and 2200 ng/g BUP-Gluc, and 42.9, 429, and 2280 ng/g NBUP-GLUC, were used to validate the method. The effect of hydrolysis on free drug was evaluated by comparing calibration curves and control samples prepared with and without enzymatic hydrolysis. The curves were coincident with no statistically significant difference in average control concentrations quantified from the two curves. Low, medium and high hydrolysis controls (n=20) were accurate within 6.6% and 7.2% of target values for BUP and NBUP, respectively. Between-run imprecision was less than 15% for both analytes at all concentrations and maximum within-day imprecision was 11.7% for BUP and 12.7% for NBUP. The following Table summarizes parameters evaluated during method validation.

	Buprenorphine			Norbuprenorphine		
Analytical Range	20 - 2000 ng/g, R^2>0.99			20 – 2000 ng/g, R^2>0.99		
Retention Time, min, mean ± SD (N=38)	5.87 ± 0.02			2.74 ± 0.01		
Control	Low	Med	High	Low	Med	High
Target Concentration (ng/g)	30	300	1600	30	300	1600
	N=20			N=20		
Inaccuracy, % difference	14.3	6.7	4.2	0.8	11.6	7.7
Between-run imprecision, % CV	8.5	8.8	9.9	12.4	4.6	6.1
	N=5			N=5		
Maximum Within-run imprecision, %CV	9.7	8.8	13.1	12.6	8.2	6.0
Extraction Efficiency, %	88.0	85.6	88.4	77.0	85.0	91.5
Matrix Suppression, %	16.8	31.3	18.5	14.0	44.9	30.0
	N=3			N=3		
Conjugate Hydrolysis Efficiency, %	91.6	77.6	91.6	74.8	89.8	99.7
Stability, % difference from fresh	N=5			N=5		
24h @ 10ºC (autosampler)	5.0	5.5	2.7	6.6	1.4	-3.6
96h @ 10ºC (autosampler)	7.2	-1.9	7.3	-2.2	3.6	-3.6
	N=3			N=3		
16h @ 22ºC (Room temperature)	-0.9	3.5	10.0	1.2	8.2	9.6
72h @ 4ºC	-4.0	-11.2	3.5	0.4	-10.5	0.3
3 Freeze/Thaw Cycles	2.0	-3.3	10.0	4.5	-3.6	-0.3
Interferences	None observed			None observed		

This is the first method to identify and quantify buprenorphine and metabolites in meconium. This sensitive and specific assay of free and total buprenorphine and norbuprenorphine will be applied to the analysis of meconium specimens collected from infants born to women enrolled in a phase II study comparing methadone and buprenorphine for treating opiate dependent pregnant women.

Keywords: **Buprenorphine, Meconium, LC-APCI-MS/MS**

P62 Comparison of Drugs of Abuse Detection in Meconium by ELISA and EMIT

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Newborns exposed to drugs of abuse *in utero* may exhibit symptoms of drug dependence and withdrawal, and suffer from short and long term health problems. Early detection can lead to effective management of withdrawal symptoms, proper treatment and a more successful outcome. Meconium is the specimen of choice for determining fetal exposure to drugs of abuse, but its complexity can create challenges when developing screening methods. Immunoassay techniques developed for urine specimens have been used successfully to detect drugs of abuse in meconium. We have used Enzyme Multiplied Immunoassay Technique (EMIT, Dade Behring) for screening of meconium specimens, but the need for lower cutoffs and higher throughput led us to consider the use of Enzyme-Linked ImmunoSorbent Assay (ELISA, Immunalysis) and an automated diluter/pipettor (TECAN Genesis).

Seventy patient specimens and nine spiked samples were used for the comparison. Samples were extracted in 7.3 pH buffer, followed by centrifugation and dilution of the supernatant with phosphate buffered saline. The calibrator for opiates was changed from morphine to hydrocodone and the calibrator for benzodiazepines was changed from oxazepam to clonazepam to maximize cross-reactivity and sensitivity for these analytes. GC/MS or LC/MS/MS techniques were used for confirmation. Results of the ELISA screening method were compared to EMIT results. The ELISA method detected more positive samples for amphetamines, cocaine, methadone, propoxyphene and opioids, and compared well to the EMIT method for THC and PCP, however the EMIT method appeared slightly more sensitive for detection of barbiturates and benzodiazepines.

Drug Class	Cutoff (ng/g)			Drug Class	Cutoff (ng/g)		
	ELISA	EMIT	Confirm		ELISA	EMIT	Confirm
amphetamine (amp)	20	200	20(GC)	opioids (opi)	20	200	2(LC)
methamphetamine (mamp)	20	200	20(GC)	oxycodone (oxy)	20	200	2(LC)
barbiturates (barb)	50	100	50(GC)	cannabinoids (THC)	20	40	10(GC)
benzodiazepines (benz)	50	100	20(LC)	propoxyphene (PXY)	50	200	10(LC)
cocaine (Be)	20	150	20(GC)	phencyclidine (PCP)	10	20	10(GC)
methadone (mtd)	25	100	10(LC)				

GC = GC/MS confirmation method; LC = LC/MS/MS confirmation method

ELISA Results Summary											
	amp	mamp	barb	benz	Be	mtd	opi	oxy	THC	PXY	PCP
Confirmed positives	11	10	7	6	24	10	25	8	37	12	9
False positives (failed to confirm)	2	1	1	2	2	1	1	2	0	0	0
ELISA positive*, EMIT negative	5	6	0	1	2	2	1	1	1	3	0
EMIT Results Summary											
	amp	-	barb	benz	Be	mtd	opi	-	THC	PXY	PCP
Confirmed positives	11	-	10	8	24	10	28	-	38	12	9
False positives (failed to confirm)	2	-	1	2	2	1	3	-	0	0	1
EMIT positive, ELISA negative	0	-	3	2	0	0	3	-	2	0	1

*screened positive but results not confirmed

Keywords: **Meconium, EMIT, ELISA**

P63 Confirmation Rates of Initial Drug Assays in a Group of SAMHSA Certified Laboratories: January 01 through December 31, 2003 vs. January 01 through December 31, 2006

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As was previously reported (SOFT 2004), the Federal Government initiated an in-depth analysis of practices in their workplace drug testing programs. Of particular interest was the specificity and cross-reactivity of the immunoassays utilized by SAMHSA (Substance Abuse and Mental Health Services Administration) Certified Laboratories. The specificity of the immunoassays has long been a subject of discussion. While it has been known that some drug class immunoassays utilized in Certified Laboratories have very high rates for the confirmation of presumptive positives, it is also recognized that other drug class immunoassays produce a significant number of presumptive positives that fail to confirm by GC/MS.

The study included data from 11 Certified Laboratories encompassing 3.9 million specimens tested under Federal mandate during 2003 and 9 laboratories encompassing 3.7 million specimens tested during 2006. These specimens represented between 50 and 60% of all federally regulated specimens tested in 2003 and 2006. The data was obtained from laboratories that utilized CEDIA, EIA and KIMS technologies as a primary initial test. In 2003 some laboratories conducted additional screening of presumptive positives with FPIA as a second initial test. Summary testing and mean confirmation rates follow. The confirmation rates are expressed as mean percent of the presumptive positives confirmed by GC/MS for each drug class and the lowest and highest laboratory confirmation rate for each drug class.

	Years	Amphetamines	BZE	Opiates	PCP	THC-COOH
Specimens Tested	2003	3,939,614	3,946,445	3,937,611	3,937,611	3,946,445
	2006	3,703,141	3,703,385	3,703,141	3.703,141	3,703,385
Presumptive Positives	2003	21,577	23,570	21,586	1,772	54,578
	2006	15,329	23,445	22,042	1,760	44,312
Confirmed Positives	2003	11,715	22,920	6,550	1,229	48,458
	2006	10,170	23,284	5,659	987	43,109

Initial Test Assay Lab Confirmation Rates	Years	Amphetamines ($1^{st}/2^{nd}$ Test)	BZE	Opiates	PCP	THC-COOH
Mean Rate	2003	51.9%/82.8%	98.1%	30.2%	69.7%	91.0%
	2006	69.2%	99.3%	24.3%	69.9%	97.7%
Lowest Rate	2003	37.4%/81.3%	91.1%	17.3%	51.6%	79.8%
	2006	53.6%	98.6%	13.6%	41.3%	94.2%
Highest Rate	2003	77.8%/84.3%	99.9%	55.9%	91.0%	98.8%
	2006	82.3%	99.8%	37.9%	98.1%	98.7%

The study evaluated the presumptive positive rates and the confirmation rates for primary initial tests by immunoassay methods (primary initial test plus second initial test in 2003). The results were examined with consideration of assay cross-reactivity and specificity. Cocaine metabolite immunoassays had the highest mean confirmation rates (>98%), followed by Marijuana metabolites (>90%), and PCP (69%). In 2006 both the "mean rate" and the "lowest rate" increased markedly for amphetamine possibly as a result of increased specificity. All confirmation rates for opiates decreased from 2003 thus remaining the lowest possibly due to the cross reactivity of other opioids.

Keywords: **SAMHSA Certified Laboratories, Immunoassay Positive Rates, Confirmation Rates**

P64 Evaluating Workplace Urine Drug Testing Results from a Medical Review Officer Data Source: 2003-2005

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Introduction: Every year a large drug testing laboratory reports indices representing roughly 7 million specimens that show an annual positive rate for both the Federally Regulated Workforce and the Non-Regulated Workforce. These annual indices may not accurately represent illegal drug use rates since they include blind quality control samples and results later reversed through Medical Review Officer (MRO) determination of valid medical explanation for the test result.

Objective: To evaluate and understand the relationships between laboratory reported drug test results and MRO verified results reported to employers over a three year period.

Methods: Records for nearly 2.7 million Regulated and Non-Regulated specimens collected during calendar years 2003 through 2005 from more than 9,000 companies and tested by 41 laboratories were obtained from a large MRO data source. The database includes donor demographics, employer information, collection site information, laboratory results, and MRO determinations, but does not include agency or employer blind quality control samples.

Results: Analysis of the data indicates that following MRO review, 9.2% of Regulated and 22.1% of Non-Regulated positive drug test results were reversed during the 36 month period. The table below illustrates the breakdown of laboratory positive specimens; MRO verified positive specimens; and MRO reversals by Regulated and Non-Regulated specimens. There was a decreasing trend of annual positive rates and an increasing trend of reversal rates in both populations. Additional analyses address specific drug classes and such issues as cancelled or rejected specimens, adulteration, and "invalid" results.

	Regulated				**Non-Regulated**			
Year	Lab +	Verified +	Reversed	# Spec. Tested	Lab +	Verified +	Reversed	# Spec. Tested
Overall 2003-05	*1.58%*	*1.43%*	*9.16%*	*428,307*	*4.18%*	*3.26%*	*22.13%*	*2,231,280*
2003	1.74%	1.64%	5.42%	164,432	4.23%	3.44%	18.53%	667,751
2004	1.51%	1.34%	11.07%	122,045	4.30%	3.36%	21.66%	736,653
2005	1.46%	1.26%	12.65%	141,830	4.05%	3.01%	25.62%	826,876

Conclusions: During the period 2003 to 2005, there was a decrease in the MRO verified positives for the Regulated (1.64% to 1.26%) and Non-Regulated (3.44% to 3.01%) specimens. An increasing percentage of laboratory positive test results were reversed in the MRO process during this period in both Regulated testing (5.42% to 12.65%) and Non-Regulated testing (18.53% to 25.62%). In the Regulated dataset, this appears to be due to a legitimate medical explanation for the presence of opiates and amphetamines. In the Non-Regulated dataset, a greater number of MRO reversals were observed largely due to higher amphetamine reversals, 43.5% versus 18.7%, and the additional testing of barbiturates and benzodiazepines, also with high reversal rates of 83.0% and 73.8% respectively.

Keywords: **Workplace Drug Testing, Database Analyses, Medical Review Officer**

P65 Comparison of TOX/See® and Triage®, Two Single-Use Cassettes for Screening Multiple Drugs in Urine Specimens

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Purpose: Compare agreement of results for two single–use cassettes for screening for panels of 8-10 drugs in urine samples. Specimens confirmed positive by GC/MS or REMEDi® (Bio-Rad Laboratories) for one drug were used.

Methods: Triage® Drugs of Abuse Panel plus Tricyclic Antidepressants is a competitive binding immunoassay. Specimen is placed in a reaction cup for 10min, transferred to a reading area, and washed. Results are read visually within 5min. Triage tests for amphetamines, barbiturates, benzodiazepines, cocaine (benzoylecgonine), PCP, opiates, TCA, and THC. The Bio-Rad TOX/See Drug Screen Test® is available in various configurations that detect different sets of drugs in urine specimens. The version tested detects the same drugs as Triage plus methadone. It also detects amphetamine and methamphetamine separately. TOX/See® is a lateral flow chromatographic immunoassay. Specimen is placed in each of four ports in the single-use device, and results are read visually at 5min. The two drug screens were compared by testing 39 urine specimens obtained commercially that had been screened positive and confirmed as positive by GC/MS, and 8 samples, obtained from a forensic laboratory, showing significant levels of TCA on REMEDi. Ten samples known to be negative for drugs on EMIT® screening tests were also tested. A total of 57 specimens plus controls were tested.

Results: Both devices gave expected results for Bio-Rad QSD Liquidchek® urine toxicology controls. All known negative specimens were negative for all drugs detected by TOX/See and Triage. TOX/See was positive for all drugs (amphetamine, methamphetamine, benzoylecgonine, PCP, opiates, benzodiazepines, barbiturates, and THC) in samples confirmed as positive by GC/MS. Triage was positive for 38 of 39; one cocaine known-positive was negative. All 8 TCA samples were positive on TOX/See and seven were positive on Triage. Additional drugs were detected in certain specimens by Triage (1) and TOX/See (7), including benzoylecgonine, PCP, opiates, and barbiturates. There are 8 drugs that are detected by both Triage and TOX/See, so that the total number of results in common for the 57 specimens was 456. The two test systems agreed in 448 of 456 qualitative determinations (98% total agreement). Of 58 results positive by at least one test, 50 were positive on both (86% positive agreement). Of 406 results negative by at least one test, 398 were negative on both (98% negative agreement).

Conclusions: TOX/See and Triage performed similarly in identifying known positive specimens. Additional drugs were detected in addition to drugs known to be present, suggesting that these multi-drug test devices are useful in detecting an array of drugs present in a single specimen. There were varying degrees of agreement between TOX/See and Triage regarding these presumptive positives. The scope of this evaluation is not sufficient to determine relative sensitivities and specificities of the test methods. TOX/See has a shorter assay time to streamline testing.

Keywords: **Tox/See, Triage, Onsite Testing**

P66 Evaluation of Gamma-Hydroxybutyrate (GHB) in Urine Specimens from a Criminal Justice Drug Testing Program

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Objective: The goal of a forensic urine drug testing program in the criminal justice system is to ascertain compliance with a defined non-drug use policy. Detection, deterrence and assessment of drug trends of prohibited drugs are the primary goals of the Cuyahoga County Department of Probation (CCP). The aim of the study was to assess the presence of gamma-hydroxybutyrate (GHB), to determine the potential of abuse by this population, and to document the endogenous and exogenous levels of GHB in adults and juveniles from a unique forensic sample pool.

Gamma-hydroxybutyrate (GHB) is an endogenous compound. A commonly regarded cerebral neurotransmitter, its applications have ranged from use as a general anesthetic, possible anabolic health adjunct for muscle building, and more recently as a recreational drug used as a "drug-facilitated sexual assault" agent.

Methods: Urine samples (n=200, adult=150, juvenile=50) were collected in 2006 over a 2 month period from individuals on probation who were subject to drug testing to monitor compliance with the non-drug use departmental policy. The ages of the subjects ranged from 12-17 years for the juveniles and 18 to 40 years for the adults. All subjects were males of varied ethnic origin. The urine specimens were stored under refrigeration in plastic containers without preservatives.

The specimens were initially screened at CCP for illicit drugs (amphetamines, cocaine metabolite, opiates, cannabinoids, and phencyclidine), creatinine and adulterants. The specimens were then transferred to CCCO for GHB testing.

The urine specimens were subjected to a modified GHB solid-phase extraction protocol from United Chemical Technologies, Inc., Horsham, PA. The extracts were derivatized with MSTFA and assayed on an HP 6890 GC interfaced with an HP 5973 MSD using a DB-5ms capillary column in the SIM mode. A 6 point calibration curve (0.5-50 mg/L) with GHB-d_6 as the internal standard and a positive control at 15 mg/L was utilized. Linearity was established between 1-150 mg/L, with a limit of detection of 0.5 mg/L. At a concentration of 15 mg/L, accuracy was determined to be 102.6%, within day precision 3.4% CV (n=10) and day to day precision 13.5% (n=10).

Results: In the adult pool the GHB concentrations ranged from 0.09 - 3.48 mg/L, with a median of 0.79 mg/L and a mean of 0.90 mg/L. The sample concentrations in the adult pool were considered “background” or endogenous. No illicit drugs screened positive in this subset. In the juvenile pool the GHB concentrations ranged from 0.34 – 3.02 mg/L, with a median of 0.75 mg/L and a mean of 0.98 mg/L (n=49). These levels were also considered endogenous. According to screening data, cannabinoids were present in 22% of the juvenile specimens with negative findings for other illicit drugs. One 15 year old juvenile was considered positive for GHB with a concentration of 25.9 mg/L. This sample screened negative for illicit drugs. The specimen was considered in excess of the administrative urinary endogenous GHB cutoff of 10 mg/L and was excluded from the mean/mean data.

For all cases (n=199) where the GHB was considered endogenous, the concentrations ranged from 0.09 – 3.48 mg/L, with a median of 0.92 mg/L, and a mean of 0.93 mg/L. There was an average of 32 days and a median of 31 days from collection to analysis of the specimens, with a range of 7-81 days.

Conclusions: In conclusion, for this particular criminal justice demographic group there was a 2% occurrence of GHB in the juvenile pool that exceeded the administrative urinary threshold. All of the other urine specimens appeared to be within acceptable endogenous levels. Further testing of GHB in a larger juvenile pool may be warranted. The endogenous levels of GHB from this unique sample population appear to correlate with previously published antemortem urine specimen ranges for GHB.

Keywords: **GHB, Criminal Justice Drug Testing, Urine Drug Testing, Endogenous**

P67 Human Urinary Elimination of 11-Nor-9-carboxy-Δ^9-tetrahydrocannnabinol (THCCOOH) by Gender

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An inpatient study of cannabis users was performed at NIDA IRP with the objective of examining the time course of THCCOOH elimination from urine. The study was conducted under the guidelines of the NIDA IRB and each volunteer gave written informed consent. Subjects resided on a closed research unit under continuous medical surveillance during cannabis abstinence, and were included if their urine at the time of recruitment tested cannabinoid immunoassay positive >100 ng/mL. Individual urine specimens were collected *ad libitum* for a period up to 30 days. The volunteers consisted of 12 male (10 African Americans, 1 Hispanic, and 1 American Indian) and 10 female (9 African Americans, and 1 Hispanic) participants. Their ages ranged from 21 to 38. All self-reported cannabis dependence or abuse and cannabis use for at least two years.

Urine specimens were analyzed for THCCOOH at LabOne-Salt Lake City under a NIDA/NIH contract. The analytical method consisted of a base hydrolysis followed by liquid-liquid extraction and THCCOOH quantification by gas chromatography/mass spectrometry (GC/MS) method with a 2.5 ng/mL limit of quantification. From demographic weight and height data collected from participants, body mass index (BMI) was calculated. The BMI ratio is a reliable indicator of body fatness and is interpreted using standard weight status categories [normal (18.5-24.9), overweight (25.0-29.0), or obese (>30.0)] applicable to adults over 20 years and both genders.

Mean ± standard deviation (SD) (range) of THCCOOH concentrations (ng/mL) in the first specimen collected upon admission for male and female subjects were similar, 286.3 ± 187.3 (31.0 - 563.0), and 247.6 ± 242.7 (56.0 - 793.0), respectively. Time of last cannabis smoking was unknown. In about half of all subjects, the maximum concentration appeared in the first specimen, and in others, within the first 30 h, similar to other published data from our group (data not shown). Mean ± SD (range) times to last consecutive positive and mean ± SD (range) THCCOOH concentrations at these times for male and female participants were 243.4 ± 110.5 h (91.3 - 408.7), 12.0 ± 4.8 ng/mL (7.0 - 26.0), and 280.7 ± 223.5 h (0.0 - 656.0), 26.2 ± 16.5 ng/mL (11.0 - 56.0) ng/mL, respectively. Mean ± SD (range) times to first negative for male and female participants were 248.0 ± 112.0 h (93.4 - 413.7) and 242.1 ± 191.2 h (0.5 - 555.2), respectively. Mean ± SD (range) total time of collection of urine specimens for male subjects was 630.8 ± 94.4 h (489.7 - 716.2), and for females 661.6 ± 78.5 h (498.7 - 729.0). Mean ± SD (range) time to last positive urine for the male participants was 491.2 ± 150.3 h (289.2 - 716.2). Mean ± SD (range) time to the last positive urine for female participants was 632.3 ± 79.6 h (498.7 - 716.0), which is almost the entire mean total time of urine collection for women participants.

Mean ± SD (range) BMI ratios for 12 male participants were 25.3 ± 3.8 (18.6 - 32.1). BMI ratios for 10 females were 29.5 ± 6.8 (22.8 - 42.5). Using the weight status charts, 7 of 12 male subjects were in the overweight or obese categories (5 overweight, 2 obese) and 7 of 10 women subjects were categorized as overweight or obese (3 overweight, 4 obese). Mean BMI's for the male and female participants in this sampling were not statistically different using a two sample t-test ($p > 0.05$). Mean time to last positive urine for female subjects is over 140 h longer than that of male subjects, which is statistically significant using a two sample t-test ($p < 0.02$). Due to its lipid nature, THCCOOH has been shown to reside in the body for extended periods of time at low but detectable concentrations when using analytical methods such as GC/MS. Higher BMI ratios in women may contribute to a longer detection window in urine. Amount and frequency of cannabis use is described by self-report only, suggesting that gender and BMI should be further evaluated under controlled conditions. These data increase our understanding of THCCOOH urinary elimination and aid interpretation of urine cannabinoid test results for men and women.

Keywords: **Cannabinoids, Excretion, Gender, Urine**

P68 Cannabichromene Exhibits Cannabinoid Activity in the Mouse Tetrad

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While Δ^9-tetrahydrocannabinol (THC) is recognized as the primary psychoactive cannabinoid in marijuana, 69 other cannabinoids, such as cannabichromene (CBC), may also have pharmacological properties or modulate the effects of THC. The degree to which these non –THC cannabinoids possess pharmacological effects independently or contribute to marijuana's overall pharmacological effects remains in question. There are reports of pharmacological benefits obtained from marijuana smoke that cannot be obtained from THC alone, which suggest that other constituents may contribute to marijuana's pharmacological effects. CBC is prevalent in most strains of marijuana in concentrations ranging from 0 – 0.65%, and few studies have investigated the effects of CBC in the whole animal.

Therefore, the present study evaluated the effects of THC and CBC in the mouse tetrad. The tetrad is a battery of assays measuring locomotor activity, catalepsy, antinociception, and hypothermia and is utilized as a screening assay for cannabinoids. ICR mice were intravenously administered single doses of THC (0.1, 0.3, 3, or 10 mg/kg) or CBC (3, 10, 30, 100 mg/kg). The mice were evaluated in the tetrad at the following post-injection time points: 5 minutes (locomotor suppression), 25 minutes (antinociception), 30 minutes (hypothermia), and 40 minutes (catalepsy). THC dose-dependently decreased locomotor activity and increased catalepsy, antinociception and hypothermia compared to vehicle control mice. CBC, at 100 mg/kg, decreased locomotor activity and increased catalepsy, antinociception, and hypothermia compared to vehicle control mice. CBC was less potent and efficacious than THC in producing cannabinoid activity across the four assays.

To determine blood and brain concentrations of THC and CBC, ICR mice received single intravenous injections of THC (0.3 or 10 mg/kg) or CBC (3 or 10 mg/kg). The mice were sacrificed and blood and brain tissue were harvested 20 minutes post-injection. THC and CBC were isolated by liquid/liquid extraction with cold acetonitrile followed by extraction with hexane/ethyl acetate (9/1). Blood and brain concentrations of THC and CBC were measured utilizing a previously validated method. Blood and brain tissue concentrations respectively were: 0.3 mg/kg THC, 2 ng/ml and 9 ng/gm; 10 mg/kg THC, 558 ng/ml and 514 ng/gm; 3.0 mg/kg CBC, 152 ng/ml and 133 ng/gm; and 100 mg/kg CBC, 10,300 ng/ml and 4,400 ng/gm. The concentrations of each cannabinoid measured were consistent with the dose response curves for THC and CBC in the tetrad. Future studies will investigate possible CBC modulation of THC in the mouse tetrad and investigate the contribution of CBC to the pharmacological effects of marijuana smoke.

Keywords: **Cannabichromene, Cannabinoids, Tetrahydrocannabinol**

P69 A Comparison of the Most Common Drugs Detected in Urine Drug Testing Samples in Different Populations in Puerto Rico

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Our objective in this paper is to evaluate the incidence of drug use in different groups tested in the Forensic Toxicology Laboratory at the Institute of Forensic Sciences of Puerto Rico. Also, a comparison is made between the percent of positive samples and type of drug detected among them.

The Urine Drug Testing Program run at the Institute tests samples from Workplace drug testing programs in state agencies as well as private companies. Also, the Program includes prisoners held in both adult and minors correctional system. Workplace and minor offender samples are routinely tested for cocaine, opiates and cannabinoids while adult prisoners are tested for cocaine, opiates, cannabinoids, amphetamines and barbiturates.

Results obtained during a period of 4 years were evaluated for the purpose of this paper. Our findings show that among the workplace population, the most prevalent drug was cannabinoids (40 %), followed by cocaine (31 %) and opiates (27 %). Among adult prisoners, the prevalence found was as follows: cannabinoids (34 %), opiates (34 %) and cocaine (29 %). In minors, the order of prevalence is cannabinoids (74 %), followed by cocaine (25 %) and opiates (1 %).

After evaluation of our statistics we concluded that cannabinoids were the most commonly encountered drug for both populations. The second most common drug varies according to the population, the incidence in prisoners is higher for opiates (in equal proportion to cannabinoids), and in minors are the second most common drug is cocaine. The third most common drug in prisoners is cocaine, and amongst minors is opiates (with a very low incidence). The incidence of drugs could be related to the time of the addicts using drugs and his/her age. In a practical sense, these statistical analyses provide important information to work with in legal proceedings, perform better controls in these populations, to improve detoxification programs and others.

Keywords: **Drugs, Prevalence, Comparison**

P70 Results of a Pilot Oral Fluids Performance Testing Program in the United States

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OBJECTIVES: Since 2000, the Center for Forensic Sciences at RTI International has been conducting a Pilot Oral Fluids (OF) Performance Testing (PT) Program for drug testing laboratories. This program was funded through the Substance Abuse and Mental Health Services Administration's National Laboratory Certification Program (NLCP) contract, with the objective of developing appropriate OF PT samples and assessing the capabilities of participating laboratories to perform confirmatory testing for drugs of abuse in OF. Examination of data from this program in 2006 led to a redesigned approach that focused specifically on within- and between-laboratory variability. This study presents the quantitative performance of laboratories participating over a period of nine months in the 2006 OF PT program.
METHODS: A total of 16 laboratories participated in the study. Of these, 14 laboratories participated for the entire study period. OF samples were manufactured in a synthetic OF matrix, and sent to the laboratories as neat, frozen 10 mL aliquots. Samples were formulated such that potentially cross-reacting drug analogs were not contained in the same sample. Three sets of samples were manufactured, with 2 sample types in each set. Set 1 samples contained methamphetamine (MAMP)/codeine (COD) and amphetamine (AMP)/morphine (MOR); Set 2 samples contained cocaine/methylenedioxyamphetamine (MDA) and benzoylecgonine (BE)/ methylenedioxyethylamphetamine (MDEA); and Set 3 samples contained tetrahydrocannabinol (THC)/phencyclidine (PCP) and 6-acetylmorphine (6-AM)/methylenedioxymethamphetamine (MDMA). The 3 sample sets were sent to the laboratory in 3 cycles, one every 3 months over the 9-month period. For each cycle, the laboratories were instructed to dilute the samples using the same dilution factor as their specific OF collection device, screen the samples once, and quantify the samples 5 times under 5 separate calibrations.
RESULTS AND CONCLUSIONS: Results demonstrated low within- and between-laboratory variability over the course of the study. For some drugs, a significant improvement was observed in analytical variability. For all drugs, the group means were very close to the targeted concentrations. Results indicated that the material was stable over the 9-month period. The table presents the group means and the group %CVs for each cycle.

Drug	*Target (ng/mL)*	*Mean Cycle 1 (ng/mL)*	*%CV Cycle 1*	Mean Cycle 2 *(ng/mL)*	*%CV Cycle 2*	*Mean Cycle 3 (ngmL)*	*%CV Cycle 3*
AMP	75	78.7	13%	76.8	13%	75.6	8%
MOR	60	60.9	20%	56.0	8%	57.6	8%
COD	60	59.1	9%	58.1	7%	58.1	9%
MAMP	75	78.0	13%	76.4	10%	75.2	8%
COC	30	30.0	10%	29.4	9%	31.1	12%
BE	30	28.7	14%	29.1	12%	28.8	14%
MDA	75	77.8	12%	76.6	10%	77.1	10%
MDEA	75	77.6	12%	78.0	8%	79.8	7%
THC	6	6.7	22%	6.0	23%	6.0	18%
6AM	6	5.8	10%	5.6	11%	5.6	11%
PCP	15	15.0	10%	14.7	13%	14.7	17%
MDMA	75	74.4	18%	75.2	9%	77.4	12%

Keywords: **Oral Fluid, Performance Testing, Drug Testing**

P71 Automated Immunoassay for the Detection of Opiates** in Oral Fluid on Roche Instrument Platforms

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A homogenous immunoassay is in development for the semi-quantitative and qualitative determination of opiates in oral fluids on Roche automated clinical analyzers**. In recent years, interest in oral fluids as an alternate matrix for measuring dugs of abuse has increased due to the ease of collection. Sample collection is less invasive than with other bodily fluids, and adulteration is more difficult. The assay utilizes KIMS technology (Kinetic Interaction of microparticles in Solution) with liquid reagents that do not require reconstitution. The two-reagent system consists of a reagent containing morphine monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. The opiates oral fluid assay utilizes a cutoff concentration of 10 ng/mL when using the Intercept®*** Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff is equivalent to approximately 40 ng/mL in undiluted oral fluid, per the proposed SAMHSA guidelines. The range of the assay is 0-80 ng/mL.

Studies demonstrate the extended stability in liquid form. When run in a semi-quantitative mode based on a six-point calibration on a Roche/Hitachi 917 analyzer, control samples at concentrations of 7.5, 10, and 12.5 ng/mL (cutoff ±25%) show mean recoveries of 7.3 ng/mL (97%), 9.8 ng/mL (98%) and 12.0 ng/mL (96%), respectively. Within run precision studies (n=21) show %CV values of 1.9%, 1.2% and 1.5%, respectively, for these control levels. The measured lower detection limit (LDL) of the assay is 0.3 ng/mL (mean + 2 standards deviations). A set of 69 clinical samples that were screened negative by the OTI OPIATES INTERCEPT® MICRO-PLATE EIA also resulted in negative values with the automated opiates assay. In addition, 91 clinical samples that screened positive by the micro-plate assay were also tested on the Roche assay and for the presence of morphine, codeine, 6-monoacetylmorphine, and hydrocodone by LC/MS/MS. Results showing agreement between the three methods will be presented. In conclusion, the assay offers accurate and reliable screening method for opiates detection in oral fluid samples on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.
***INTERCEPT is a trademark of ORASURE Technologies, Inc.

Keywords: **Oral Fluid, Opiates, Immunoassay**

P72 Automated Immunoassay for the Detection of Cocaine** in Oral Fluid on Roche Instrument Platforms

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A homogeneous immunoassay is in development for the semi-quantitative and qualitative determination of cocaine metabolite in oral fluids on Roche automated clinical analyzers**. In recent years, interest in oral fluids as an alternate matrix for measuring dugs of abuse has increased due to the ease of collection. Sample collection is less invasive than with other bodily fluids, and adulteration is more difficult. The assay utilizes KIMS technology (Kinetic Interaction of microparticles in Solution) with liquid reagents that do not require reconstitution. The two-reagent system consists of a reagent containing a benzoylecgonine monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. The cocaine oral fluid assay utilizes a cutoff of 5 ng/mL when using the Intercept®*** Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoff concentration is equivalent to approximately 20 ng/mL in undiluted oral fluid, per the proposed SAMHSA guidelines. The range of the assay is 0 - 40 ng/mL.

When run in a semiquantitative mode with a 6-point calibration on a Roche/Hitachi 917 analyzer, control samples at concentrations of 3.75, 5.0, and 6.25 ng/mL (cutoff ± 25%) show mean recoveries of 3.9 (104%), 5.0 (99%), and 6.2 (99%), respectively. Within run precision studies (n=21) show %CV values of 2.0 %, 2.5% and 1.4%, respectively, for these control levels. The measured lower detection limit (LDL) of the assay is 0.3 ng/mL (mean + 2 standard deviations). The assay is specific to benzoylecgonine with cross reactivity of less than 0.6% to cocaine, ecgonine and ecgonine methyl ester. A set of 110 clinical samples that screened negative with the OTI Cocaine Metabolite INTERCEPT® MICRO-PLATE EIA were also screened negative (>99%) with the Roche assay.

In summary, the assay produce accurate and reliable results and is well suited for routine screening of cocaine metabolite in oral fluids.

**These assays are currently in development and have not been approved for use in the US by the FDA.
***INTERCEPT is a trademark of ORASURE Technologies, Inc.

Keywords: **Oral Fluid, Cocaine, Immunoassay**

P73 Automated Immunoassays for the Detection of Amphetamines and Methamphetamines** in Oral Fluid on Roche Instrument Platforms

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New homogeneous immunoassays are in development for the detection of amphetamines and methamphetamines in oral fluids on Roche automated clinical analyzers. The assays consist of a two reagent systems. In recent years, interest in oral fluids as an alternate matrix for measuring dugs of abuse has increased due to the ease of collection. Sample collection is less invasive than with other bodily fluids, and adulteration is more difficult. The assay utilizes KIMS technology (Kinetic Interaction of microparticles in Solution) with liquid reagents that do not require reconstitution. The amphetamine and methamphetamine/MDMA reagents are separate assay systems. Each assay is a two-reagent system consisting of a reagent containing a monoclonal antibody that is covalently linked to carboxy-modified polystyrene microparticles, with a drug-polymer conjugate in solution as the second reagent. Both the amphetamine and methamphetamine oral fluid assays utilizes cutoff concentrations of 12.5 ng/mL when using the Intercept®*** Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI). The cutoffs are equivalent to approximately 50 ng/mL in undiluted oral fluid, per the proposed SAMHSA guidelines. The range of each assay is 0-100 ng/mL.

The performance of these assays were tested on the Roche/Hitachi 917 automated analyzer. Within run precision studies on the amphetamine assay were performed by running 21 replicates of controls in the semi quantitative mode on the Hitachi 917. Control samples at 9.4 and 15.6 ng/mL recovered mean values of 9.7 (103%) and 15.6 ng/mL (100%), respectively, with respective %CV values of 3.8% and 1.6%. Cross-reactivities to PMA, and MDA were 124%, and 80% respectively. The measured LDL (limit of detection) was 0.6 ng/mL at 2 standard deviations. Within run precision studies on the methamphetamine assay (n=21) showed that control samples at 9.4 and 15.6 ng/mL methamphetamine recovered mean values of 9.5 (101%) and 15.1 ng/mL (97%), respectively, with respective %CV values of 3.3% and 2.5%. Cross- reactivities to MDEA, MDMA and PMMA were 89%, 103%, and 108%, respectively. The measured LDL (limit of detection) was 1.0 ng/mL at 2 standard deviations. A set of 100 clinical samples that screened negative with the OTI Amphetamines and Methamphetamines INTERCEPT® MICRO-PLATE EIA were also screened negative (99%) with the Roche assays.

The methamphetamine and amphetamine assays offer accurate and reliable screening methods for the detection of amphetamines and methamphetamines in oral fluid samples on automated systems.

**These assays are currently in development and have not been approved for use in the US by the FDA.
***INTERCEPT is a trademark of ORASURE Technologies, Inc.

Keywords: **Amphetamines, Oral Fluid, Immunoassay**

P74 Analysis of Opiates in Oral Fluids Utilizing LC/MS/MS

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Objective: The objective of this paper is to develop a method for a simple, quick method for analysis of opiates in oral fluids.

Methods: Analytes included in this method are: 6-Monoacetyl Morphine (6-MAM), Codeine, Morphine, Oxycodone, Hydrocodone, and Hydromorphone. Oral fluid samples were collected on an Intercept® Oral Fluid Drug Test collection device. 400µl of oral fluid is collected on the collection device which is then placed into 800µl of preservative solution resulting in a total volume of 1200µl. Using 400µl of this solution for sample preparation results in a 1:3 dilution of the oral fluid sample. Liquid-liquid extraction using Methylene Chloride, Isopropanol, and 1% HCl in Methanol was used for sample clean-up. The samples were extracted by LLE then evaporated to dryness under a stream of Nitrogen before reconstitution in mobile phase.

LC/MS/MS analysis was performed on an HPLC stack interfaced to a hybrid triple quadrupole/linear ion trap mass spectrometer. Separation was achieved on a Phenomenex Gemini C_{18} column (2.1 X 50mm, 5µm) using water and methanol as the mobile phases. Formic acid (0.1%) was added to each and a rapid gradient was used to elute the compounds. Analytical run time was under 10 minutes.

Results: Liquid-liquid extraction proved to be a simple and effective extraction and clean-up. Extraction efficiency was greater than 75%. Because LC/MS/MS analysis was utilized, no further derivatization was necessary. The lower limit of quantitation was 4 ng/mL for all analytes except 6-MAM which had a LOQ of 0.4 ng/ml.

Conclusion: A quick, simple method was developed to analyze opiates in oral fluids. Testing of oral fluid for detection of illicit drug use is becoming increasingly popular due to: non-invasive sample collection; “gender neutral” collection; and lower probability of adulteration. LC/MS/MS has the selectivity and sensitivity to detect the presence of opiates at the low levels found in oral fluid. Sample preparation was also greatly simplified versus other analytical techniques and total analytical run time was under 10 minutes.

Keywords: **Oral Fluid, Opiates, LC/MS/MS**

P75 A Sensitive and Selective Method for Detection and Quantitation of 6-acetylmorphine by GC/MS Equipped with an Oral Fluid Analyzer

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The ability to confirm the presence of 6-acetylmorphine (6-AM) in urine is essential for differentiating morphine positive samples of heroin users from those who have been administered morphine or use codeine. This is required in federally mandated testing, where the Substance Abuse and Mental Health Services Administration (SAMHSA) has determined that the federally certified Laboratories must be able to quantitate 6-AM down to at least 4 ng/mL (40% of the cutoff), even in the presence of interfering compounds such as other opiates. Oxycodone and Oxymorphone have especially been problematic, requiring bisulfite treatment, solid phase extraction, longer chromatographic columns or a combination of these methods and other procedures to resolve.

A simplified method has been developed utilizing a liquid/liquid extraction followed by analysis on the Agilent 6890/5975 GC/MS equipped with an oral fluid analyzer (Dean's switch with cryofocuser). The method requires no bisulfite pretreatment but still eliminates interferences by redirecting the effluent long enough to capture the 6-AM from column one (DB-1) into the more polar column two (DB-17) via the Dean's switch. The samples are derivitized with BSTFA to form TMS derivatives prior to analysis by GC/MS.

The method has shown the ability to easily quantitate down to 4 ng/mL 6-AM in the presence of 10,000 ng/mL codeine, morphine, norcodeine, hydrocodone, hydromorphone, oxycodone and oxymorphone. The LOD/LOQ for the assay is 1 ng/mL, with a ULOL of 300 ng/mL, although greater limits can be achieved. The precision was measured at the cutoff (10 ng/mL) and at 40% (4 ng/mL) with both CVs under 5%.

In conclusion, this method provides highest sensitivity and selectivity for the detection and quantitation of 6-AM in urine and can be used to detect 6-AM in oral fluids.

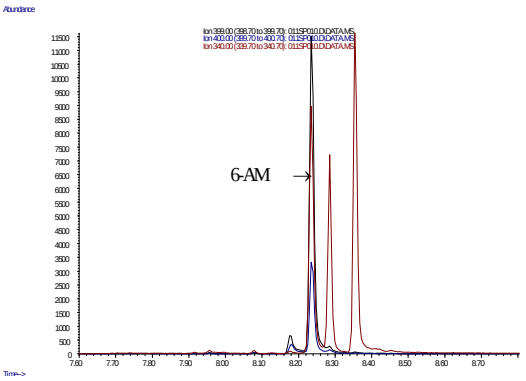

Figure 1. Ion chromatogram of a 4 ng/mL 6-acetylmorphine (6-AM) sample spiked with 10,000 ng/mL oxycodone.

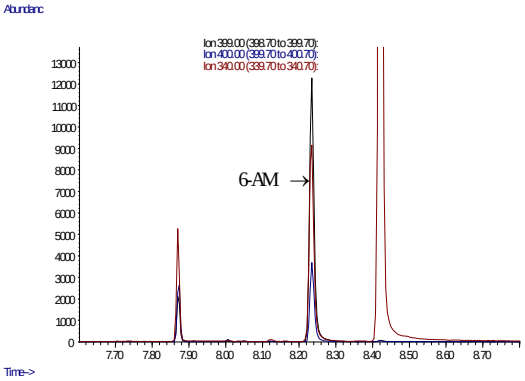

Figure 2. Ion chromatogram of a 4 ng/mL 6-acetylmorphine (6-AM) sample spiked with 10,000 ng/mL oxymorphone.

Keywords: **6-AM, GC/MS Oral Fluid Analyzer, Drug Testing**

P76 A Rapid GC-MS Determination of Gamma-Hydroxybutyrate (GHB) in Saliva

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GHB and related compounds have been known for years in the forensic toxicology because of their illicit use in drug-facilitated sexual assault (DFSA) and to a lesser extent, as party drugs. This problem is exacerbated by GHB's rapid clearance rate and short half-life of ~30 min. For this reason, it would be useful to develop a rapid screening analysis from a biological matrix that predictably tracks plasma drug concentration. Oral fluids, which can be collected non-invasively, are an attractive option. Unfortunately, saliva drug concentrations are generally significantly lower than those in urine, which creates challenges for method development.

Taking these factors into consideration, we have developed a sensitive and specific gas chromatography-mass spectrometer (GC-MS) method using selective ion monitoring (SIM) for the identification and quantification of gamma-hydroxybutyric acid (GHB) in saliva. In this approach, 1.0 µl of synthetic saliva was spiked with 1.0µl of GHB-d6 as the internal standard. As an added quality assurance method 1.0 µl of 1,7-heptanediol is added to all samples as a surrogate spike. The purpose of the surrogate is to track the efficiency of extraction and preparation procedures. This provides an additional degree of control over matrix effects given that recovery of the surrogate compound is anticipated to track that of the target compound; here, GHB. Internal standard and surrogate spike concentration was adjusted to obtain the final amount of 10.0 ppm.

After a silyl-derivatization the sample was injected at a split ratio of 10:1. The following ions were monitoring: GHB 233, 234; GHB-d6: 239, 240, 241; 1,7-heptanediol: 55, 73, 97. No interferent peaks were observed. The LOQ was determined to be 0.5 ppm with a linear dynamic range of 0.5 ppm to 50 ppm. Quality-control samples (5 ppm, 20 ppm, 30 ppm) were prepared for evaluation of analytical precision found to be from 1.07 to 9.44% in both intra-day and day-to -day experiments. Surrogate recovery from saliva samples fell in the range of 94.6 to 100% with an average of 98.37% and a corresponding % RSD of 1.2%.

Blank samples from lab staff were analyzed to estimate endogenous GHB in saliva. Values in the range of 2-3 ppm were typical, although the number of samples was relatively small (n=7 to date). GHB concentration was found to be in the concentrations range from less than LOQ to 29 ppm and inter-day variations are currently being studied. Results will be included in this presentation. We are currently analyzing post mortem swabs from West Virginia State Toxicologist and are seeking additional case samples. These results will be presented.

The described method can be proposed for rapid, selective and accurate toxicological screening saliva analysis for forensic purposes. The use of a surrogate standard provides a quantitative measure of extraction and preparation efficiency that is matrix specific. The method described here could be applied to swabs, neat saliva, and possibly physical evidence such as saliva on drink glasses. Current research is focused on the latter application.

Keywords: **GHB, GC-MS, Oral Fluid**

P77 Methadone Maintenance Program: Utility of Oral Fluid

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Background: Methadone is used in the treatment of heroin addiction as part of a compliance program, however on occasion, patients will add a small amount of methadone to a urine specimen in order to prove intake, then sell the remainder on the street. An observed collection, perhaps using oral fluid, for methadone testing may help circumvent this problem. However sample volumes are limited and oral fluid typically contains lower concentrations of drugs than urine, so a rapid, simple specific homogenous immunoassay (HEIA) has been developed for the detection of methadone in oral fluid samples obtained with the Quantisal™ collection device. The assay format is compatible with most commercially available chemistry analyzers.

Validation: The immunoassay uses 15 µL of the collected specimen (1 mL of neat oral fluid diluted in 3 mL transportation buffer in the Quantisal™ device). The intra-assay precision at 25, 50, 75 and 100 ng/mL of methadone was determined to be 0.86%, 0.74%, 1.41% and 0.96%; inter-assay precision was 0.81%, 0.9%, 1.03% and 0.69% respectively. The detection limit of the screen was 5 ng/mL. The assay was further challenged with oral fluid specimens previously confirmed by GC-MS at a cut-off of 50 ng/mL.

ELISA	GC-MS	
	+	-
+	27	1
-	0	55

Sensitivity: 27/(27)=100%; *Specificity*: 55(1+55)=98.2%; *Accuracy*: >98%

Cross-reactivity: There was no significant cross-reactivity with other opiates, which are reportedly present in the urine of up to 30% of methadone patients. Other medications approved for the treatment of heroin including levo-alpha- acetyl-methadol (LAAM) and its metabolite nor-LAAM showed a cross-reactivity of 55% and 50% respectively. Other common drugs of abuse were spiked in negative oral fluid and showed no cross-reactivity in the assay. None of these drugs were found to interfere with the detection of methadone in the assay.

Summary: The described HEIA method is sensitive, specific and precise for the detection of methadone in oral fluid. Oral fluid is a convenient matrix for observed sample collection. The assay format is compatible with most commercially available chemistry analyzers.

Keywords: **Homogeneous Immunoassay, Methadone, Oral Fluid**

P78 Detection of Dextromethorphan and its Metabolite Dextrorphan in Oral Fluid

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Background: Dextromethorphan (DXM) is an antitussive, which is being extensively abused by teenagers across the USA. Dextromethorphan hydrobromide is the major ingredient found in a variety of over the counter cough syrups such as Robitussin®. From 1999 to 2004, the California Poison Control System reported a 10-fold increase in DXM abuse cases, with the median age of abusers being sixteen. DXM has 3 metabolites, the major one being dextrorphan (DXO). Previous ELISA methods have been developed which detect DXM and DXO, but in separate assays. We describe a unique and sensitive ELISA method, which detects both DXM and DXO in the same assay in oral fluid. The method was applied to specimens collected from subjects using the Quantisal™ device, following voluntary ingestion of DXM.

Results: Specimens collected before and after a therapeutic dose of DXM (15-30 mg)

	Subject 1			**Subject 2**			**Subject 3**		
	ELISA	*GCMS (ng/mL)*		*ELISA*	*GCMS (ng/mL)*		*ELISA*	*GCMS (ng/mL)*	
Oral Fluid	P/N**	DXM	DXO	P/N	DXM	DXO	P/N	DXM	DXO
Pre-dose	N	0	0	N	0	0	N	0	0
3 hrs	P	5.5	7.8	P	5.7	4.9	P	76.6	10.9
6 hrs	P	4.7	4.7	P	2.8	3.1	P	74.8	11.3
12 hrs	P	1.6	2	N	1.5	1.8	P	58	9.7
24 hrs	N	1.3	1.4	N	1.5	1.6	N/A*	N/A*	N/A*
48 hrs	N	0.9	1.4	N	0.9	1.4	N/A*	N/A*	N/A*

*no specimens collected after 24 hrs; **P/N: positive or negative (cutoff conc = 4 ng/mL)

DXM and DXO were detected in all subjects up to 48 hours after a single oral therapeutic dose.

Validation: The intra and inter-day precision by ELISA were found to be <10% for both drugs.
Confirmation of positives by ELISA was carried out using GC-MS. The detection limit by ELISA and GC-MS for both drugs was 1 ng/mL. The percentage recovery of the drugs from the pad in the Quantisal™ collection device was 93.1% for DXM and 93.9% for DXO. Both methods were challenged with authentic oral fluid specimens (*n*=16) from volunteers, and good correlation was obtained between them.

ELISA	GC-MS	
	+	-
+	7	1
-	0	8

Sensitivity: 7/(7)= 100%
Specificity: 8(8+1)= 88.9%
Levorphanol, the isomer of DXO does not cross-react with this ELISA assay.

Summary: The described methods are sensitive, specific and precise for the detection of DXM and DXO in oral fluid. Oral fluid is a convenient matrix for use in high school situations, where observed collection can be easily carried out. These methods can be applied to the detection of DXM in the toxicology field as part of a teenage drug of abuse test panel.

Keywords: **Dextromethorphan, ELISA, GC-MS, Oral Fluid**

P79 Analysis Of Benzodiazepines In Oral Fluid Using The Quantisal™ Collection Device And LC/MS/MS

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Background: Oral fluid is a useful biological specimen to detect recent usage of drugs and has specific advantages over urinalysis. However, the concentration of several benzodiazepines in oral fluid is very low, and sample volume is limited. A rapid, simple, highly sensitive procedure for the simultaneous analysis of 14 benzodiazepines: bromazepam, alprazolam, clonazepam, lorazepam, oxazepam, diazepam, midazolam, flurazepam, flunitrazepam, nordiazepam, triazolam, temazepam, nitrazepam and chlordiazepoxide, using solid phase extraction and a liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC/MS/MS) is described.

Sample Collection: Oral fluid samples were obtained using the Quantisal™ collection device, which provides 1 mL of neat saliva diluted with transportation buffer (3 mL).

MS Instrumentation: An Agilent Technologies 6410 liquid chromatograph LC/MS/MS operating in positive electrospray mode was used. The column was a Zorbax Eclipse XDB C18 (4.6mm x 50mm x 1.8 μm). The column temperature was held at 35^{o}C; the initial flowrate was 0.2 mL/min and the injection volume was 5 μL. Solvent A consisted of 20mM ammonium formate; solvent B was acetonitrile. The mobile phase composition remained at 50:50 (v,v). The flowrate was held at 0.2 mL/min for 6.5 min then increased to 1mL/min. After 8 minutes, the flowrate was decreased back to 0.2 mL/min. The total run time was 10 minutes, with a 4.5 min post-run allowing mobile phase equilibrium. The MS operated at a capillary voltage 4500V; nebulizer pressure 50 psi; nitrogen gas flow 6 L/min and gas temperature 300^{o}C.

Validation: Using multiple reaction monitoring, two transitions were analyzed and one ion ratio determined, which was within 20% of the ratio for known calibration standards. The limits of quantitation ranged from 0.5 – 5ng/mL of neat oral fluid; the intra-day precision of the assays (n=5) ranged from 2.8 -7.29%; and the inter-day precision ranged from 1.42 - 6.8% (n = 5). The percentage recovery of the drugs from the collection pads ranged from a low of 81.4% for midazolam to 90.17% for nitrazepam.

Discussion: The software was able to monitor a secondary transition from the precursor ion and automatically calculate the ratio to the primary ion. If the ratio was not within 20% of a calibration standard, the identification was rejected. Monitoring a second transition gives additional confidence in the result; applying a ratio to that second transition compared to the primary product ion is a further enhancement to the identification of drugs in oral fluid. The software plots the ratio in the chromatographic window, so the operator is able to assess positivity visually. This is important in forensic analysis where court challenges to laboratory data are frequent.

Summary: The described assay is precise, specific and sensitive, and is suitable for the analysis of 14 benzodiazepines in oral fluid collected with the Quantisal™ device.

Keywords: **Benzodiazepines, LC/MS/MS, Oral Fluid**

P80 Forensic Identification of Cocaine and Metabolites in Hair using LC/MS/MS: Importance of Dual Transition Monitoring in Tandem Mass Spectrometry

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Background: Cocaine (COC) and its metabolites are included in the proposed Federal regulations for hair analysis. The cut-off concentration for the metabolites is 50 pg/mg, which is difficult to achieve routinely using GC/MS. This may be due to the inability to derivatize cocaethylene (CE) to improve its response; the co-elution of norcocaine (NC) and CE, or potentially similar ions for the derivatives of NC and benzoylecgonine (BZE). Therefore, we developed and validated a procedure using LC/MS/MS to circumvent these problems and improve the durability of our assay. Two transitions for each drug were monitored so a ratio between a quantitation and a qualifying ion could be calculated, in order to provide additional confidence in the generated result. This is particularly important in assays that include compounds with similar molecular weights and chemical properties, since the same product ion is often present.

Methods: Hair specimens, both drug free and from authentic users were included in our protocol. For validation, drug free hair specimens (10 mg) were fortified with cocaine and its metabolites at 25, 50, 100, 200, 500, 1000, 2000 and 10,000 pg/mg. Each of the hairs was incubated, extracted, evaporated to dryness and reconstituted in methanol.

LC/MS/MS Conditions: An Agilent LC 1200 Series pump connected to a 6410 tandem MS operating in positive APCI mode was used. The column was an Eclipse XDB C18 (4.6 x 50mm x 1.8um), held at 40^{o}C. The injection volume was optimized at 2uL, which gave adequate response and improved precision over 5uL injections. The mobile phase consisted of 20mM ammonium formate, pH 6.4 (A) and methanol (B). The phase was held at 70:30 for 1.5 min, then raised to 55%B at 4.5 min (flowrate 1 mL/min); 60% B after 5 min and 75%B after 7 min. The gas temp was 350^{o}C; gas flow was 5 L/min; nebulizer pressure was 50 psi and capillary 4500 V. The fragmentor was set at 120V for all transitions except D8-CE (160V). The LOD was 25 pg/mg and the assay was linear to 10,000 pg/mg. Inter and intra-day precision was less than 10% and l6% respectively.

Compound	*Precursor ion*	*Product ion*	*CE (V)*	*Qualifier range ratio (%)*
D3-BZE	293.3	171.2	20	
BZE	290.3	168.3 (105.3)	15 (15)	6.7 – 10
D3-Cocaine	307.3	185.3	20	
Cocaine	304.3	182.3 (82.2)	20 (25)	37.8 – 56.8
D8-CE	326.3	204.4	20	
CE	318.3	196.4 (82.2)	25 (25)	49.3 - 74
D3-NC	293.3	171.2	15	
NC	290.3	168.3 (136.3)	15 (25)	17.4 – 26.1

Summary: The assay is suitable for the confirmation of COC and metabolites at the proposed Federal concentrations. The use of a qualifying ion improves the validity of the identification and increases confidence in the final result.

Keywords: **Tandem Mass Spectrometry, Cocaine and Metabolites, Hair**

P81 Stability and Reproducibility of Amphetamines and THCA in a Pilot Hair Performance Testing Program

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Objectives: Since 2000, the Center for Forensic Sciences at RTI International has been conducting a Pilot Hair Performance Testing (PT) Program for drug testing labs. This program is funded through the Substance Abuse and Mental Health Services Administration's National Laboratory Certification Program (NLCP) contract, with the objectives to develop appropriate hair samples for a PT program and assess the capabilities of participating labs to perform analytical testing for drugs of abuse in hair. This study presents the quantitative performance of hair labs participating over a 1 year period in the 2006-2007 Pilot Hair PT program.
Methods: A total of 8 labs participated in the study. Of these, only 3 to 5 reported results for the entire study period depending on the analyte. Hair samples were either manufactured by fortifying drug negative human hair with drugs of abuse or were procured from drug users. Samples were formulated with 1 drug class in each sample. Three sets of hair samples were manufactured, with 1 to 2 drug sample types in each set. Set 1 samples contained amphetamines [amphetamine (AMP), methamphetamine (MAMP) methylenedioxyamphetamine (MDA) methylenedioxyethylamphetamine (MDEA) methylenedioxymethamphetamine (MDMA)] and delta-9-carboxylic acid tetrahydrocannabinol (THCA); Set 2 samples contained cocaine analytes and Set 3 samples contained phencyclidine and opiate analytes. This presentation shows data for the first sample set containing amphetamines and THCA. The 3 sample sets were sent to each lab in 4 cycles, 1 every 4 months over the 12-month period. The hair PT samples were sent to the labs as 150 mg aliquots. The labs were instructed to analyze the samples without decontamination. Labs then confirmed the samples 5 times under 5 separate calibrations for each directed analyte of interest. Hair samples were analyzed by each lab up to 20 times over the 12 month study period.
Results And Conclusions: Within-lab variability was lower for the amphetamines (%CV range was 4-34%, mean, 19%) than for THCA (%CV range was 15-33%, mean, 27%). Table below shows group means and the group %CVs for each cycle and that between-lab variability decreased for labs that were able to report results for the entire study period for a given drug. For most drugs, the group means across cycles were close to the targeted concentrations. Results indicated that the hair materials were stable over the 12 month period.

A random effects model ANOVA was also run to assess differences in Lab and Cycle variation. For all of the hair analyses, the interaction between Lab and Cycle variation was found to be significant (p-value < 0.05). These data indicate that as a PT system the amount of lab variability was not consistent across cycles or across labs.

Drug	*# of Labs*	*Target (pg/mg)*	*Mean Cycle 12 (pg/mg)*	*%CV Cycle 1 2*	*Mean Cycle 15 (pg/mg)*	*%CV Cycle 15*	*Mean Cycle 18 (pg/mg)*	*%CV Cycle 18*	*Mean Cycle 21 (pg/mg)*	*%CV Cycle 21*
TIME INTERVAL			*0 months*		*4 months*		*8 months*		*12 months*	
AMP	5	900	834	31.3	782	29.3	946	13.6	906	11.5
AMP	5	450	397	38.9	463	21.9	436	20.3	449	11.6
MAMP	5	900	854	30.8	825	29.1	991	15.6	934	11.0
MAMP	5	450	411	40.1	493	22.6	469	22.0	481	13.7
MDA	5	900	857	31.4	831	28.3	991	15.8	908	12.7
MDA	5	450	421	42.2	460	21.7	467	18.2	455	11.6
MDMA	3	900	718	33.0	729	31.8	860	16.3	829	13.9
MDMA	3	450	316	39.3	397	24.0	399	19.7	408	15.0
MDEA	5	900	648	35.7	791	24.1	872	16.7	841	7.7
MDEA	5	450	278	36.2	432	18.8	398	22.1	412	14.2
THCA	3	0.82	0.96	28.0	0.75	29.8	0.60	33.0	0.68	21.7
THCA	3	0.15	0.17	28.2	0.16	45.5	0.15	24.2	0.13	24.6

Keywords: **Hair, Performance Testing, Drug Testing**

P82 Studies of Four Extraction Methods for Recovery of Opiates from Hair

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Four published methods for extraction of opiates from hair were studied: (A) enzymatic digestion using proteinase K and dithiothreitol (1); (B) pulverization followed by sonication for 3 hours in methanol at 37°C (2); (C) incubation in pH 2.9 phosphate buffer/0.1% BSA at 60°C for 1 hour (3); and (D) incubation in acidified methanol for 1 hour at 60°C (4) or 2 hours at 70°C. For the studies of methods A, B, C, and D, fifteen, seven, eleven and eleven different hair samples were analyzed, respectively.

All hair samples were first subjected to an extended aqueous washing procedure before extraction to minimize presence of drugs on the hair from environmental contamination or sweat of users. After recovery from the hair by the four procedures, the drug in the extracts was further purified using Cerex Polychrom PSCX solid phase extraction columns and analyzed by liquid chromatographic-tandem-mass spectrometry (LC/MS/MS).

Recoveries of over 90% of morphine (MOR), codeine (COD), 6-acetylmorphine (MAM), and oxycodone (OXY) were obtained in 6 hours digestion at pH 6.65 and 37°C. Average recoveries by sonication in methanol of pulverized hair at 37°C were 53.9% of COD, 56.5% of MOR and 67% of MAM (SD's 11.2, 8.3, and 6.2%, respectively). Average recoveries with the phosphate buffer method were 17.8% of COD, 15.0% of MOR, 22% of MAM (S.D.'s 7.4, 6.5, 13.8%, respectively). Average recoveries after an hour heating at 60°C in acidified methanol method produced average recoveries of 4.1% of COD, 3.0% of MOR, and 8.8% of MAM (SD's 5.2, 2.0, and 6.3%, respectively).

Experiments were also performed on the effects of chopping and pulverizing hair on recovery of drugs with the above three solvent methods. Results with chopped hair were essentially similar to those with whole hair for all of these methods, while pulverizing the hair increased recoveries significantly.

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Keywords: **Opiates, Hair, Extraction**

P83 A General Screening Procedure For Drugs In Hair

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The analysis of hair for the detection of xenobiotics has gained widespread attention in the forensic community during the last decade. Many compounds and their metabolites were already reported in hair and segmental analysis provided useful information on the profile of drug intake (acute vs chronic). Unfortunately, multiple factors have to be considered before a sound interpretation: irregular hair growth rate, various incorporation mechanisms, cosmetics, contamination etc. Most papers being concerned by a single compound or a family of drugs like amphetamines, opiates or others, our laboratory wanted to develop a method to screen a wide range of substances in hair.

The objective of this study was to design a general screening procedure for hair samples and compare results with post-mortem blood to determine if some correlation could be made; therefore a series of postmortem cases were examined. Considering the confounding factors mentioned earlier, our objective was not to quantitate the amount of drug nor its precise time of use, but to establish past usage and the general trend in those investigated cases.

Cardiac or femoral blood and hair cut close to the skin from the posterior vertex sampled at autopsy at the Laboratoire de sciences judiciaires et de médecine légale de Montréal, were analysed in 35 different cases. General blood screening was done first and in positive cases in which sufficient hair was available, hair analysis was performed. After washing procedures, all samples were extracted by solid phase extraction using Oasis cartridges, reconstituted in ethanol and analysed by GC/NPD/MS for general drug screening and LC/MS/MS for opiates, cocaine and metabolites, amphetamines, cannabinoids and benzodiazepines.

In the majority of cases, the wash procedure seemed adequate. As already noted in previous papers, basic drugs seem better incorporated into hair than neutral, acidic or polar substances. Most drugs found in blood were positive in hair making it likely to assume chronic use. However in overdose cases, drugs found in blood were not present in hair which could presume a single event. Segmental hair analysis being an important tool to determine chronic or in some cases a single previous exposure without often knowing the substance involved, we needed to be able to perform a general screen on this type of matrix.

Keywords: **Screening, Hair, Blood**

P84 Detection of a Prescription Diuretic in the Over-the-Counter Dietary Supplement StarCaps®

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Diuretics are used clinically to treat hypertension and to reduce peripheral or pulmonary edema resulting from congestive heart failure or kidney disease. Athletes may use diuretics, as masking agents, to increase urine production and thereby decrease urinary concentrations of banned performance enhancing agents such as anabolic steroids. Thus, the use of diuretics by athletes is prohibited by sporting federations such as the International Olympic Committee (IOC). It was brought to the attention of our laboratory that StarCaps®, an over-the-counter dietary supplement, might contain the prescription diuretic "bumetanide". StarCaps® is marketed as an "all-natural" dietary supplement containing papaya and garlic extracts and no amphetamine, nicotine, thyroid, caffeine or ephedrine. Directions for use include taking a glass of orange juice, a banana, or potassium with 1 StarCaps capsule. Bumetanide is a loop diuretic which inhibits $Na^+/K^+/2Cl^-$ transport system in the ascending limb of the loop of Henle, thereby inhibiting NaCl reabsorption and enhancing urinary excretion of NaCl as well as K+. Toxicity of loop diuretics, such as bumetanide, includes hypokalemic metabolic acidosis which can be reversed by K+ replacement. Typical therapeutic dosages of bumetanide are 0.5-2 mg daily. The purpose of this study was to determine whether StarCaps® contained bumetanide or other banned diuretics, and if persons taking an acute dose of StarCaps® would test positive for a banned diuretic. Two male and two female volunteers were administered a single StarCaps® capsule and their urine specimens were collected at 2, 4, 8, and 12 hrs post-administration. Following solid phase extraction, urine specimens were analyzed by high performance liquid chromatography-mass spectrometry (HPLC/MS) for 19 banned diuretics. Bumetanide was the only banned diuretic identified, and was detected in all specimens at concentrations ranging from 4.6 to 351.3 ng/mL. Peak concentrations occurred at 8 hrs post-administration. StarCaps® capsules (n=5) were analyzed by HPLC/MS for banned diuretics and found to contain bumetanide at concentrations consistent with therapeutic doses (0.74 to 0.87 mg/capsule). The presence of bumetanide in urine samples and StarCaps® capsules was confirmed using HPLC-quadrupole/time-of-flight mass spectrometry. These results demonstrate that unregulated dietary supplements put both athletes and consumers at risk of unwitting consumption of prescription medications that may result in disciplinary actions against athletes who test positive. Additionally, overzealous use of such products may potentially result in severe dehydration or other adverse clinical effects in unsuspecting users.

Keywords: **Supplement, Bumetanide, StarCaps**

P85 Look Mom No Hands: Toxicology Testing in the Automated Clinical Laboratory

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Traditionally, clinical toxicology testing was performed in a specialized laboratory or with specific instrumentation separate from that used to perform chemistry or other clinical testing. With the development of immunoassays and very recent advances in laboratory automation, toxicology may now be fully incorporated into the routine clinical laboratory workflow. We present our experience with the performance of "Stat" toxicology testing as part of an automated clinical laboratory system. Urine drug screening is by immunoassay testing for amphetamines, barbiturates, benzodiazepines, cocaine metabolite, methadone, methaqualone, opiates, phencyclidine, and propoxyphene. Serum drug screening is by immunoassay testing for barbiturates, benzodiazepines, cocaine metabolite and opiates with quantitative analysis for salicylate, and acetaminophen. Quantitative serum immunoassays for specific drugs such as digoxin, lidocaine, phenobarbital and phenytoin may be easily added to these panels. All assays are performed on the automated system.

Once received in the laboratory, specimens are logged into a Cerner laboratory information system (LIS). Serum specimens are loaded directly onto the Labcell (Siemens Diagnostics) laboratory automation system (LAS) by a robotic sample manager. Aliquots of urine are poured into specified tubes and loaded onto the LAS. The sample manager places the tubes on a continuously moving track that carries them to a series of chemistry and immunochemistry autoanalyzers. Barcode reader stations automatically divert the samples to the appropriate analyzer for testing. Once the analyzer removes an aliquot of sample, the samples are diverted back onto the main track to be carried to other analyzers for additional testing or moved and stored by the sample manager. All toxicology testing is performed on Advia 1650 Chemistry System auto-analyzers (Bayer Health Care, Diagnostics Division). The entire process is only a few minutes in duration, with results available for review within ten to twelve minutes.

A vendor specific LIS system (Centralink) allows review of all results before releasing them to the ordering hospital site or agency. Over a sixteen-month period, urine drug immunoassays controls of -25% and +25% of the cut-off concentration (Bio-Rad) yielded CV's <10%. Serum drug immunoassay controls of –50% and +50% typically yielded CV's <10%.

In summary, laboratory automation is becoming the mainstream technology for large medical institutions. The Labcell LAS allows for the incorporation of toxicology testing into the routine workflow of the clinical laboratory, resulting in consistent turn around times in STAT toxicology testing to support emergency departments with a minimum of specimen handling.

Keywords: **Laboratory Automation, Stat Toxicology, Labcell**

P86 Application of Fast GC-MS in the Analysis of Opiates

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GC-MS analyses have been made faster by using shorter columns, reducing column internal diameter, increasing carrier gas flow rate and faster temperature ramps in a conventional oven. Usually, these changes result in loss of chromatographic resolution. Currently we are employing a 220 volt power supply and an oven shroud that allow temperature ramps up to 120 degrees per minute. In our opiate method a fast temperature ramp was applied at the beginning of the run up to just before the first analytical peak and again just after the last analytical peak until the end of the run. A very high temperature ramp was not applied during the analyte peak window and chromatographic resolution was not reduced. GC-MS analysis was performed on Hewlet Packard 6890 gas chromatograph equipped with 5973 mass selective detector. HP-5MS column, 15 meter, 0.250 mm I.D, 0.25 μm film thickness was used for separation of opiates. Helium was used as a carrier gas at a constant flow of 1 ml/minute. The injector temperature was 250 °C. The transfer line was maintained at 280°C. The column was ramped from an initial 50°C to 200°C at a rate of 80°C/minute. A second ramp was set from 200°C to 270°C at 20°C/minute. The last step was from 270°C to 310°C at 80°C/minute, with a total run time of 7.88 minutes.

Opiates (hydrocodone, codeine, morphine, hydromorphone) and their respective deuterated internal standards (d3) spiked in blood were extracted using Detectabuse extraction columns, type-GV-65 from Biochemical Diagnostics, Inc., and derivatized with propionic anhydride.

This fast GC-MS method for opiates was linear from 0.05 to 1.5 mg/L with correlation coefficient of 0.99 for each analyte. Analysis of 6 replicate blood controls spiked with 0.10 mg/L of hydrocodone, codeine, hydromorphone and morphine gave average determinations of 0.09, 0.10, 0.11, and 0.10 mg/L respectively with coefficient of variation (CV %) of 8.2, 7.4, 9.9 and 9.6. Similarly analysis of 6 replicate blood controls spiked with 1.0 mg/L of hydrocodone, codeine, hydromorphone and morphine gave average determinations of 0.89, 1.12, 1.07, and 1.08 mg/L, respectively with CV % of 5.5, 8.8, 9.2, and 7.8. By this “fast” GC/MS method, analytical run time was reduced by 50 percent, without loss of assay performance.

Keywords; **Fast GC-MS, Opiates**